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THE PHARMACOLOGY OF PSYCHOTOMIMETIC AND PSYCHOTHERAPEUTIC DRUGS

BY

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Conference Chairman and Consulting Editor
SEYMOUR S. KETY

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THE CONFERENCE AND ITS AIMS

By Seymour S. Kety
National Institute of Mental Health and National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

In November 1955 I was invited by The New York Academy of Sciences to organize a conference on *The Interactions of Lysergic Acid Diethylamide and Serotonin.* The Academy readily agreed to broaden the scope of the conference to include much of the newly awakened interest in psychopharmacology, but the choice of a suitable title constituted a somewhat difficult problem. We wanted the conference to deal with the pharmacology of those drugs that act primarily upon the mind or, more exactly, those drugs that affect mental state. The most suitable designation that I have heard for these drugs is that of Walter Loewe, who called them "phrenotropic drugs." Unfortunately, this term has not received the wide familiarity that it deserves and, for this reason, it was felt that the designation would have to be somewhat more explicit. The designation "psychotomimetic agents" for those drugs that mimic some of the mental aberrations that occur in the psychoses had been suggested by Ralph Gerard and seemed especially appropriate. The term "ataractic drugs" that Howard Fabing devised for the tranquilizing agents would have been preferable to "psychotherapeutic drugs." In any case, *The Pharmacology of Psychotomimetic and Psychotherapeutic Drugs* was the title finally chosen for the conference. If not brief, at least it was fairly explicit and it is upon that conference that this publication is based.

It was not easy to select from the large number of investigators who have contributed significantly to this field the small number who might adequately represent the field in the presentation of formal papers. Even though we confined the presentations almost exclusively to papers dealing with the pharmacology of this class of agents, we knew that we were certain to overlook some major contributions. The presentations were planned in such a manner that the longer papers would review the field and, in that way, would increase the comprehensiveness of the conference and of this monograph. To complement these general reviews of the subject a number of shorter papers appear that report work currently in progress or recently completed. It will be seen that these papers represent only an introduction to each of the fields touched upon and that, in the discussions of the individual papers, opportunities were taken to present new information and new concepts.

I acknowledge with appreciation the support and assistance of the Division of Behavioral Sciences of the Ford Foundation, New York, N. Y., which, by means of a grant to The New York Academy of Sciences, has aided in making this publication possible.

Part I. Psychotomimetic Agents: Clinical and Biochemical Aspects

A REVIEW OF THE CLINICAL EFFECTS OF PSYCHOTOMIMETIC AGENTS*

By Humphry Osmond
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The Psychotomimetic Agents

We are using Gerard's[25] term "psychotomimetics" generically for compounds that have been called schizogens, psychotica, psychotogens, phantastica, hallucinogens, and elixirs. If one believes that the importance of these compounds lies in their capacity to mimic the mental illnesses called psychoses, psychotomimetics would be the term of choice. This capacity alone would make these drugs the focus of psychopharmacological inquiry, but this capacity is not their only, nor even, perhaps, their most important quality. It is but recently that the relationship between psychotomimetics and schizophrenia has become somewhat clearer. Later in this presentation I shall suggest a more inclusive term.

We are the latest of generations of experimenters who, from before the dawn of history, in every part of the world, have sought for means by which man could alter, explore, and control the workings of his own mind, thus enlarging his experience of the universe. Until recently, however, science has shown only sporadic interest in these matters.

Since there are few substances that, in large enough doses, will not produce changes in body and mind resembling some mental illness, I shall try to limit our field of inquiry by a definition that will prevent workers in this field from being overwhelmed with an inconveniently large portion of the pharmacopoeia. This definition will be improved, and I make it in order to provoke such improvement: "Psychotomimetic agents are substances that produce changes in thought, perception, mood and, sometimes, in posture, occurring alone or in concert, without causing either major disturbances of the autonomic nervous system or addictive craving, and although, with overdosage, disorientation, memory disturbance, stupor, and even narcosis may occur, these reactions are not characteristic."

This rough and ready guide excludes morphine, cocaine, atropine, and their derivatives. It also excludes anesthetics, analgesics, and hypnotics. I expect, however, that there will still be some overlapping of these divisions. Using this guide we have a sizable list, starting with the still-unidentified soma[21] imported from central Asia into India several thousand years ago and ending with 3,4,5,trimethoxyphenyl-β-aminopropane (TMA),[55] bufotenin,[73] adrenochrome,[31] and adrenolutin[34, 53] whose qualifications for inclusion in this classification are still being examined. As I list these treasures of 5000

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years of perilous and sometimes fatal searching, think upon those nameless discoverers and rediscoverers, Aztec and Assassin, Carib and berserker, Siberian and Red Indian, Brahmin and African, and many others of whose endeavors even scholars do not know. We inherit their secrets and profit by their curiosity, their courage, and even from their errors and excesses. Let us honor them. They do not appear in any list of references.

There are such substances as soma, hashish, cohoba, ololiuqui, peyote, the Syrian rue, the caapi vine, the fungus teonanacatl, the two Amanitas, pantherina and muscaria, the iboga bean, and the fierce virola snuff obtained from a nutmeglike tree in Amazonia. Who knows what other compounds await the keen inquiries of ethnobotanists such as R. E. Schultes or mycologists such as Gordon Wasson?

With our modern synthetics we are a little safer, though the ground quakes beneath us. These synthetics include mescaline,[28] introduced by Heffter in 1896, the first, I believe, of these agents to be synthesized; harmine or telepathine,[54] an alluring name whose significance I have never understood; Hoffman's[70] astonishing lysergic acid diethylamide (LSD), whose great activity has made homeopathy seem less improbable; hashish, whose still-uncertain active principles should surely be ascertained; TMA, synthesized by Scott and his co-workers of Imperial Chemicals Ltd., Manchester, England, a synthetic that lies in an area intermediate between mescaline and amphetamine and has recently been the subject of a report by J. R. Smythies; bufotenin, isolated from cohoba, a West Indian snuff; unstable adrenochrome; and the subtle adrenolutin. What an array of substances for daring inquiry! What work for generations to come!

We know little enough about the most familiar of these agents, and there are only vague correlations between the physical and mental changes that they cause. Considering their interest to medicine alone, our lack of information is disquieting, but they are of more than medical significance. They reach out to psychology,[50, 64] sociology,[56] philosophy,[66] art,[58, 75] and even to religion.[37, 38, 65] Surely we are woefully ignorant of these agents and this ignorance must be remedied.

To clinicians such as myself who daily encounter those crippling illnesses that confine hundreds of thousands of unhappy people to dismal and obsolete institutions flattered by the name of hospital, publication of a monograph such as this one is heartening. Perhaps it means that apathy and neglect are ending, and we may have a chance to apply the immense but often unused knowledge that we already possess regarding the care of the mentally ill, and that we shall get encouragement and support to seek for even more.

In What Way Are These Substances Important?

Nearly everyone who works with psychotomimetics and allied compounds agrees there is something special about them. Such words as "unforgettable" and "indescribable" abound in the literature. Few workers, however, have emphasized that the unique qualities of these substances must be investigated in many directions at the same time, a consideration that makes work in this field all the more difficult. I shall try to remedy this deficiency by citing several reasons for ascribing importance to them, although in the attempt I am sure to show my scientific shortcomings and imaginative limitations.

(1) The primary interest of these drugs for the psychiatrist lies in their capacity to mimic more or less closely some aspects of grave mental illnesses, particularly of schizophrenia. The fact that medical men have been preoccupied with transient states resembling mental illnesses that have been called model psychoses, however, does not mean that the only use for these compounds is in the study of pathological conditions. This misunderstanding, unless corrected, can deprive us of much knowledge and prevent the growth of new and fascinating researches. Model psychoses allow us to correlate human experience with animal behavior. We can learn how to aggravate and alleviate these model illnesses, and thus we can devise "model therapies" that may later have wider application.

(2) Psychiatrists have found that these agents have a place in psychotherapy. This practice may sound like carrying the idea of "a hair of the dog that bit you" rather far, but it seems to be justified.

(3) Another potentiality of these substances is their use in training and in educating those who work in psychiatry and psychology, especially in understanding strange ways of the mind.

(4) These drugs are of value in exploring the normal mind under unusual circumstances.

(5) Last, but perhaps most important: there are social, philosophical, and religious implications in the discoveries made by means of these agents. To inquire more than superficially into any one of them would require more time than I have available and more knowledge and wisdom than I possess.

The Model Psychoses

Over a century ago B. A. Morel, according to Ellenberger,[14] used hashish to show his students the sort of world that might be endured by some mentally ill people. It is remarkable how ignorant we still are as to the best way to conduct such experiments. We still do not know for certain the exact differences between experiences produced by, for example, hashish, and those produced by peyote, peyote and mescaline, or mescaline and LSD. Of course such comparisons have been made, but I know of none that has taken into account obvious variables such as body type, height and weight, or skin and eye color, let alone subtle personality or cultural, social, and biochemical factors that may be very important. We are still unsure whether these substances differ quantitatively or qualitatively. Our work with ololiuqui,[53] adrenochrome,[31] and adrenolutin[34, 53] has caused me to suspect that these drugs are qualitatively different from mescaline and LSD. The Aztecs who first used ololiuqui held the same opinion. I have had a few reports of the results obtained from using mixtures of these drugs, but I am unaware of any established facts about them.

From all accounts,[15, 45, 65] the cactus peyote is unpleasant to take, with the result that the isolation and synthesis of mescaline by Heffter[28] encouraged more work to be done. Beringer,[6] Rouhier,[59] Klüver,[45] G. Tayleur Stocking,[72] Mayer-Gross,[49] and Paul Hoch[30] should be mentioned in this connection. The introduction by Hoffman[69] of LSD and subsequent investigation of this compound by Stoll[71] added an immensely potent weapon to our armory. The minute concentration of it required to produce its effect and the fact that, according to Cerletti,[10] most of the drug is excreted from the body within 1 hour while its effects last 12 hours or more is an unsolved mystery. How does the drug continue to act although it is no longer present?

Among many excellent papers on LSD, apart from Stoll's[71] original reports, observations by Rinkel, Hyde, and Solomon[57] and by Anderson and Rawnsley[2] are outstanding.

It is curious that in the lengthy and sometimes heated discussions about the relationship of model psychoses to schizophrenia that smoldered for nearly 50 years, not until 1951[52] was the difference between a transient, artificially induced, experimental state in a volunteer under laboratory conditions and the prolonged, insidious, creeping illness in an unsuspecting victim whose social life progressively atrophied, clearly recognized.

There is one golden rule that should be applied in working with model psychoses. One should start with oneself. Unless this is done, one cannot expect to make sense of someone else's communications and, consequently, the value of the work is greatly reduced. Stefaniuk[68] told me how much his attitude changed after he had himself taken LSD during the course of a series of experiments.

I am still unsure in what way patients should participate in these investigations. Rinkel[58] has observed that mentally ill patients can be made worse by LSD. One cannot be dogmatic, but an investigator might ask himself whether someone who cannot communicate much at best is likely to be very useful during an experience that often silences healthy volunteers who have agreed to do their ulmost to report. Changes in behavior can be noticed, but one questions their value when their meaning is obscure.

After Smythies[52] rediscovered the similarities in the structural formula of mescaline and adrenalin we started to hunt for substances that might be psychotomimetic and that lie in the enormous series between the 2 compounds. We thought that we should find some of those intermediate compounds whose activity would be nearer to that of adrenalin than that of mescaline. Clinical information from asthmatics and from Asquith,[3] an anesthetist, directed our attention to adrenalin that has lost its pressor qualities. We first investigated adrenochrome and, later, adrenolutin. We think that these 2 compounds are psychotomimetics. Fabing[17] has recently carried out trials with bufotenin.

While our work has still to be repeated in humans by independent investigators, work with animals[63] has been encouraging. An early attempt by Rinkel, Hyde, and Solomon[57] to reproduce our work with semicarbazone, a more stable adrenochrome, failed, which suggests that there is something specific in the unstable molecule. Later attempts with adrenochrome from another source have also been unsuccessful. The trouble seems to spring from difficulties inherent in the synthesis of both adrenochrome and adrenolutin.

A recent letter from J. Harley Mason of Cambridge University, England, states that the causes of these difficulties are not yet clear. I hope that this will be a challenge to organic chemists to sort out and classify these indolic derivatives of adrenalin whose very instability makes them excellent prospects for use as natural psychotomimetic agents. The changes that they induce, although sometimes very striking, are more subtle and less florid than those induced by mescaline or LSD. Consequently, these changes are harder to detect, delineate, and measure and, for persons who are used to mescaline and LSD, they may seem very small. We are trying, however, to reproduce a cross section of an illness that is insidious, that seeps into its victim over a period of weeks and months, so that these characteristics that make the experiment so difficult to perform are perhaps encouraging.

The more our psychotomimetics resemble the hypothetical endotoxin that Carl Jung[43] called toxin-X and that we have called *M* (mescalinelike) substance, the harder they will be to test and the more attention they will require in experimental design. Since someone already loaded with *M* substance might not respond to it at all, it would seem wise in this type of experiment to exclude psychotics, neurotics, epileptics, alcoholics, and psychopaths and to rely on normal volunteers. Isbell's[40] demonstration of the tolerance that is developed for a short time after taking LSD supports this suggestion.

There are other difficulties, such as finding regular supplies of a particular agent, uncertainties about the proper route for administration, individual differences in absorption and susceptibility, the dearth of subjects skilled in self-observation, and the effects of placebo on both the observer and the observed. Before all this comes the task of designing testing schedules to measure and correlate physiological, electrophysiological, biochemical, psychological, and social changes, and then the task of relating these changes to a naturally occurring illness—schizophrenia. The work bristles with difficulties, yet it must be done, for the rewards that it offers are large, even at the soberest reckoning.

No account of model psychoses would be complete that did not relate those that are induced chemically to those induced by other means, such as the reduced or specialized environments described by Heron, Bexton, and Hebb[29] and by Lilly.[46] These specialized environments have been used since antiquity, and they raise a host of questions, one of which is of sufficient urgency to discuss briefly.

Most people can adjust themselves to small changes in perception quickly enough for these changes to be of no importance. There are a few situations in which even these small changes can be dangerous and, unless they are expected and sought for, they might not be recognized. In high-speed flying and as they will in any flight into outer space, men suffer major psychophysiological and psychochemical changes within a very short time (it is artificial to separate these 2 categories, but the division is practically useful). We know that damage to the liver, even though it has been sustained many years previously, may prolong the effects of both mescaline[22] and adrenochrome[31] by hours and even days in some people. Under great stress, in the special environment of both the pressure suit and the cabin of an aircraft, a pilot's liver, affected by changes in gravitational pull and possibly by anoxia, might not work sufficiently well to detoxicate the by-products of his own adrenalin. If this happened the pilot might not be able to exert sufficient control to survive.

An aggravating factor in these circumstances would be the steady drinking of alcoholic liquor, due to its known tendency to damage the liver. Low blood sugar and inadequate niacin intake would certainly increase these tendencies. Coffee and tobacco, in excess, would increase the danger.

Atropine derivatives especially should be avoided by men flying planes. It has long been known that hashish[42] and dhatura make a very deadly mixture that may well enhance endogenous psychotomimetics. Tonini[75] has shown that Methedrine will prolong the effects of LSD-25 and will also intensify them. Thought must be given to lights flashing at certain frequencies[36] and goggles[47, 76] or cabin covers of particular colors that may be necessary at great altitudes.

With so many possible factors to take into consideration, much patient investigation is needed, and one suspects that many unexpected changes in perception would escape notice simply because they cover so many fields.

Do such changes actually occur? I do not know. I do know, however, that the only occasion on which I have ever been unable to relate time to distance was after taking adrenochrome. The reaction made it impossible to drive a car, and it even made being driven very unpleasant. I wonder what it would have been like trying to land a jet plane?

Uses in Psychotherapy

I have read Sandison's,[60] Abramson's,[1] and Frederking's[23] accounts of the use of LSD-25 and mescaline in psychotherapy, but I have not been able to see the reports of Busch and Johnson.[8] I have done some work in this field myself, and I have access to the records of a colleague who has conducted an extended series of experiments in psychotherapy with these substances. Let me emphasize again that those who have not themselves taken the particular substance with which they wish to work, preferably several times, would be wise not to use these agents in therapy. Possibly no one has done this, but no paper that I have read has made it the essential precondition for such work.

Abramson,[1] using a modified psychoanalytical approach, gives small doses of LSD-25 in repeated sessions. He aims to resolve early conflicts by abreaction, free association, and re-education. Sandison[60] gives a varying dose of LSD-25 to chronic neurotic patients in a mental hospital. He uses the experience for group discussion and psychotherapy of a Jungian nature. Frederking,[23] whose account is the most sophisticated, compares mescaline and LSD-25, and he discusses about 200 treatments. He uses psychoanalytical methods. Our work started with the idea that a single overwhelming experience might be beneficial to alcoholics, the idea springing from James[41] and Tiebout.[74] Thus far it seems that a high dose may be valuable, but that repeated treatment is necessary. At this stage of our investigations we have not yet observed enough patients to be able to give any hard and fast rules as to prescribing these drugs.

Hubbard,[36] whose large unpublished series of cases has been most kindly placed at my disposal, has treated a number of gravely ill alcoholics. All seem benefited to some extent, and a number of them to a degree that the patients themselves consider miraculous. Looking over the records it is hard not to agree with the appraisal these patients gave of themselves.

All new therapies enjoy an early period of high success, so that cautious optimism seems to be our wisest attitude, yet there are exciting, indeed, extraordinary possibilities available to the therapist who has himself endured these experiences. The substances in question can be used to develop very high degrees of that mysterious yet vital quality—empathy. Shall we find a means by which the therapist will share, to a far greater degree than he commonly does now, his patient's experience? Freud,[24] Jung,[44] and many others[11] have long been aware of transient happenings of this sort.

We should perhaps do well not to be too hidebound by old techniques when using these new tools.

Psychotomimetics and Training

I know of no study dealing specifically with the application of these substances to the training of the workers engaged in many different disciplines who work together in psychiatry. Such training has resulted from experimental work, but only incidentally. Hyde[39] and others have used these substances to enlarge the sympathy of members of a psychiatric staff for patients in their care. Such a journey of self-discovery may one day be obligatory for those working in psychiatry. Although it might not always be pleasant, with care and understanding this experience would be very useful to the trainee.

The Model Therapies and the Reverse

Schueler[61] seems to have been the founder of model therapies when, in 1934, he gave some of his mescalinized medical students sodium succinate by vein. This treatment reduced their symptoms briefly, but the symptoms recurred when the succinate was excreted, which happened quickly. Mayer-Gross,[48] using the LSD 25 model, showed that perceptual change is reduced when the blood sugar is over 200 μg. per cent. Elkes[13] found that both chlorpromazine and sodium amytal antagonised LSD. Fabing[16] altered the LSD-25 model with azacyclonal (Frenquel), but with mescaline it seems that the effects recurred after more than one half-hour. Hyde,[39] during the course of some elegant work, discovered that a social setting that is protective and nutritive results in a reduction of paranoid tendencies and perceptual changes. Hoffer and Agnew[33] used nicotinic acid to alter the LSD-25 model. Giberti and Gregoretti[26] used both reserpine and chlorpromazine in LSD-25 models, and Schwarz, Bickford, and Rome[62] found that LSD-25 models and 1 mescaline model were much reduced by chlorpromazine.

Schueler's[61] work has never, I think, been used in psychiatric treatment on any scale, although Smythies[67] used sodium succinate on a few chronic schizophrenics without any change. Mayer-Gross's[48] work may have a bearing on insulin therapy and, if it does, a radical change in technique seems indicated. It is said that schizophrenia and diabetes rarely occur together. This may be a lead worth following. Chlorpromazine, reserpine, and Frenquel have, of course, all been used extensively in therapy. Hoffer and I, with our associates,[32] are preparing a paper on a large series of schizophrenics treated with massive doses of niacin. At this stage, the results seem promising.

Ways of aggravating model psychoses are equally important, though we know little about them. Hyde[39] found that cold and, particularly, inquisitorial attitudes increase perceptual disorders and paranoid trends. Atropine and its derivatives aggravate the effects of hashish[42] and also those of schizophrenia.[35] The effect of dhatura on hashish has been known for many years in India, and is said to have been used by professional robbers in that country to produce temporary madness in their victims. Methedrine, as I have already indicated, prolongs and reactivates the LSD-25 model. According to a drug addict, benzedrine in large doses, dissolved in black coffee, is very like mescaline in effect. Hubbard[36] reports that 30 per cent CO_2 and 70 per cent oxygen will both exaggerate and reactivate the LSD-25 model when inhaled. Smythies,[67] using a stroboscope and, latterly, a variable-speed shutter, finds that this enhances some aspects of the mescaline model. I have mentioned earlier in this paper that some people who have had infective hepatitis many years previously endure greatly prolonged responses to mescaline and adrenochrome.

I should like to see these models combined with a reduced or specialized environment. We also need to know within what limits hypnosis can eliminate, aggravate, and facilitate these psychic changes.

Psychotomimetic Agents and Psychology

Heinrich Klüver[45] has pioneered so many trails that it will be no surprise to discover that nearly 30 years ago he was emphasizing the importance of mescaline to psychology in an admirable book now unhappily out of print.

The advances in our understanding of the hallucinatory, the illusional, and the delusional that Klüver considered could be made by studying the effects of mescaline and similar experiences are, for the most part, still undone. During an experiment with adrenochrome I found myself an "it," a thing. The sensation was not one of unreality. It might be called "depersonalization," but I am not sure that a great variety of self-perceptions is not subsumed under that label. Only comparison and careful classification will tell us.

Let us consider empathy, that feeling for, in, or with other creatures or even things that seems to be so poorly described in psychological texts. Yet when it is lacking to any great degree something essentially human is lost. Empathy, I know, can increase until one is "involved in mankinde," something that most of us feel only when deeply in love. Saints have had such experience sustained for a lifetime, but for the rest of us a few moments of it are ever remembered as supreme exaltation. When members of the Native American Church,[65] peyote takers, say that in their meetings this happens frequently, I believe them. It may seem unlikely that the usually insensitive can become acutely and exquisitely aware of the feelings of others, but they can do so.

The development of synesthesia, that strange fusing of 2 or more sensory modalities, has received some attention, but we know little about minor degrees of this sort of perception and the problems of communicating them. How might this affect schizophrenics? Bleuler[7] in an account of the existentialist psychotherapy gives a hint of the possibilities here.

There is also the matter of thought blocking. I have noticed 3 different varieties of this phenomenon that appear similar to an outside observer. In one of them, so great is the press of associations that they rupture the chain of thought. In another, illusions and even hallucinations distract one's attention. In the third type it is as if the power of concentration fails and thought fades out. This third type seems to spring from a physiological level. Those who have been able to see *in* themselves will, I believe, devise better means of inquiry than we now possess.

For the social psychologist there are group studies springing from observations already made with peyote takers. We have made tentative explorations of the experiences of group use of LSD-25. The effects are strange and impressive. We seem to have almost no language suitable for communicating them. It is as if new dimensions of human relationship are revealed. Such work can be done only by those who are used to these substances.

Other Inquiries Arising from This Work

It is encouraging that many are joining in the hunt now that the question of psychotomimetic indoles has been raised again. Federoff,[18, 19, 20] at the University of Saskatchewan, is pursuing the matter of blood toxicity in schizophrenia, with interesting results. We need much more detailed information about these derivatives of adrenalin and other related compounds, so that we can obtain regular supplies of those that have known, predictable psychotomimetic activity. At the moment there seems to be a considerable difference in potency between various batches of these drugs, and no one knows the precise reason. There is an urgent need for vigorous research in this area of psychopharmacology.

We also need a means of temporarily enhancing the effect of psychotomimetics so that while avoiding a model psychosis we can nevertheless spot their presence in the volunteer. Smythies,[67] using the stroboscope, has given us a valuable clue here, and he is following this lead at Cambridge University, Cambridge, England.

If we are to succeed, close cooperation between many varied disciplines will be necessary.

I hope that foundations, governments, and large firms will keep this thought continually in mind. Let us encourage people from distant and often hostile groups to meet, talk, and listen together. Let us lure them into making those essential friendships. The attempt will be worth it.

The Exploration of Experience

Our interest, so far, has been psychiatric and pathological, with only a hint that any other viewpoint is possible, yet our predecessors were interested in these things from quite different points of view. In the perspective of history, our psychiatric and pathological bias is the unusual one. By means of a variety of techniques, from dervish dancing to prayerful contemplation, from solitary confinement in darkness to sniffing the carbonated air at the Delphic oracle, from chewing peyote to prolonged starvation, men have pursued, down the centuries, certain experiences that they considered valuable above all others.

The great William James[41] endured much uncalled-for criticism for suggesting that in some people inhalations of nitrous oxide allowed a psychic disposition that is always potentially present to manifest itself briefly. Has our comparative neglect of these experiences, recognized by James and Bergson[5] as being of great value, rendered psychology stale and savorless? Our preoccupation with behavior, because it is measurable, has led us to assume that what can be measured must be valuable, and vice versa. During the 20th century we have seen, except for a few notables such as Carl Jung, an abandoning of the psyche by psychologists and psychiatrists. Recently they have been joined by certain philosophers.[4] Pavlov, Binet, Freud, and a host of distinguished followers legitimately limited the field to fit their requirements, but later expanded their formulations from a limited inquiry to embrace the whole of existence. An emphasis on the measurable and the reductive has resulted in the limitation of interest by psychiatrists and psychologists to aspects of experience that fit in with this concept.

There was and is another stream of psychological thought in Europe and in the United States that is more suitable for the work that I shall discuss next. James, in the United States, Sedgwick, Myers,[51] and Gurney in Britain, and Carl Jung in Switzerland are among its great figures. Bergson[5] is its philosopher and Harrison[27] its prophet. These and many others have said that in this work, as in any other, science is applicable if one defines it in Dingle's[12] term, "the rational ordering of the facts of experience." We must not fall into the pitfall of supposing that any explanation, however ingenious, can be a substitute for observation and experiment. The experience must be there before the rational ordering.

Work on the potentialities of mescaline and the rest of these agents fell on the stony ground of behaviorism and doctrinaire psychoanalysis. Over the years we have been deluged with explanations, while observation has become less sharp. This will doubtless continue to be the case as long as the observer and the observed do not realize that splendor, terror, wonder, and beauty, far from being the epiphenomena of "objective" happenings, may be of central importance.

Accounts of the effect of these agents, ranging in time from that of Havelock Ellis[15] in 1897 to the more recent reports of Aldous Huxley,[37, 38] are many, and they emphasize the unique quality of the experience. One or more sensory modalities combined with mood, thinking and, often to a marked degree, empathy, usually change. Most subjects find the experience valuable, some find it frightening, and many say that it is uniquely lovely. All, from Slotkin's[65] unsophisticated Indians to men of great learning, agree that much of it is beyond verbal description. Our subjects, who include many who have drunk deep of life, including authors, artists, a junior cabinet minister, scientists, a hero, philosophers, and businessmen, are nearly all in agreement in this respect. For myself, my experiences with these substances have been the most strange, most awesome, and among the most beautiful things in a varied and fortunate life. These are not escapes from but enlargements, burgeonings of reality. In so far as I can judge they occur in violation of Hughlings Jackson's[40a] principle, because the brain, although its functioning is impaired, acts more subtly and complexly than when it is normal. Yet surely, when poisoned, the brain's actions should be less complex, rather than more so? I cannot argue about this because one must undergo the experience himself. Those who have had these experiences know, and those who have not had them cannot know and, what is more, the latter are in no position to offer a useful explanation.

Is this phenomenon of chemically induced mental aberration something wholly new? It is not, as I have suggested earlier. It has been sought and studied since the earliest times and has played a notable part in the development of religion, art, philosophy, and even science. Systems such as yoga have sprung from it. Enormous effort has been expended to induce these states easily so as to put them to use. Although occasionally trivial and sometimes frightening, their like seems to have been at least part of the experience of visionaries and mystics the world over. These states deserve thought and pondering because until we understand them no account of the mind can be accurate. It is foolish to expect a single exploration to bring back as much information as 20 of them. It is equally foolish to expect an untrained, inept, or sick person to play the combined part of observer, experiencer, and recorder as well as a trained and skilled individual. Those who have no taste for this work can help by freely admitting their shortcomings rather than disguising them by some imposing ascription.

This may seem mere nonsense but, before closing his mind, the reader should reflect that something unusual ought to seem irrational because it transcends those fashionable ruts of thinking that we dignify by calling them logic and reason. We prefer such rationalized explanations because they provide an illusory sense of predictability. Little harm is done so long as we do not let our sybaritism blind us to the primacy of experience, especially in psychology.

Psychoanalysts claim that their ideas cannot be fully understood without a personal analysis. Not everyone accepts this claim, but can one ever understand something one has never done? A eunuch could write an authoritative book on sexual behavior, but a book on sexual experience by the same author would inspire less confidence. Working with these substances, as in psychoanalysis, we must often be our own instruments.

Psychoanalysis resembles Galileo's telescope, which lets one see a somewhat magnified image of an object the wrong way round and upside down. The telescope changed our whole idea of the solar system and revolutionized navigation. Psychotomimetic agents, whose collective name is still undecided, are more like the radar telescopes now being built to scan the deeps of outer, invisible space. They are not convenient. One cannot go bird watching with them. They explore a tiny portion of an enormous void. They raise more questions than answers, and to understand those answers we must invent new languages. What we learn is not reassuring or even always comprehensible.

Like astronomers, however, we must change our thinking to use the potentialities of our new instruments.

Freud has told us much about many important matters. However, I believe that he and his pupils tried illegitimately to extrapolate from his data far beyond their proper limits in an attempt to account for the whole of human endeavor and, beyond this, into the nature of man and God. This was magnificent bravado. It is not science, for it is as vain to use Freud's system for these greatest questions as it is to search for the galaxies with Galileo's hand telescope. Jung, using what I consider the very inadequate tools of dream and myth, has shown such skill and dexterity that he has penetrated as deep into these mysteries as his equipment allows. Our newer instruments, employed with skill and reverence, allow us to explore a greater range of experience more intensively.

There have always been risks in discovery. Splendid rashness such as John Hunter's should be avoided, yet we must be prepared for calculated risks such as those that Walter Reed and his colleagues took in their conquest of yellow fever. The mind cannot be explored by proxy. To deepen our understanding, not simply of great madnesses but of the nature of mind itself, we must use our instruments as coolly and boldly as those who force their aircraft through other invisible barriers. Disaster may overtake the most skilled. Today and in the past, for much lesser prizes, men have taken much greater risks.

How Should We Name Them?

If mimicking mental illness were the main characteristic of these agents, "psychotomimetics" would indeed be a suitable generic term. It is true that they do so, but they do much more. Why are we always preoccupied with the pathological, the negative? Is health only the lack of sickness? Is good merely the absence of evil? Is pathology the only yardstick? Must we ape Freud's gloomier moods that persuaded him that a happy man is a self-deceiver evading the heartache for which there is no anodyne? Is not a child infinitely potential rather than polymorphously perverse?

I have tried to find an appropriate name for the agents under discussion: a name that will include the concepts of enriching the mind and enlarging the vision. Some possibilities are: psychephoric, mind-moving; psychehormic, mind-rousing; and psycheplastic, mind-molding. Psychezymic, mind-fermenting, is indeed appropriate. Psycherhexic, mind bursting forth, though difficult, is memorable. Psychelytic, mind-releasing, is satisfactory. My choice, because it is clear, euphonious, and uncontaminated by other associations, is psychedelic, mind-manifesting. One of these terms should serve.

Epilogue

This, then, is how one clinician sees these psychedelics. I believe that these agents have a part to play in our survival as a species. For that survival depends as much on our opinion of our fellows and ourselves as on any other single thing. The psychedelics help us to explore and fathom our own nature.

We can perceive ourselves as the stampings of an automatic socioeconomic process; as highly plastic and conditionable animals; as congeries of instinctive strivings ending in loss of sexual drive and death; as cybernetic gadgets; or even as semantic conundrums. All of these concepts have their supporters and they all have some degree of truth in them. We may also be something more, "a part of the main," a striving sliver of a creative process, a manifestation of Brahma in Atman, an aspect of an infinite God imminent and transcendent within and without us. These very different valuings of the self and of other people's selves have all been held sincerely by men and women. I expect that even what seem the most extreme notions are held by some contributors to these pages. Can one doubt that the views of the world derived from such differing concepts are likely to differ greatly, and that the courses of action determined by those views will differ?

Our beliefs, what we assume, as the Ames[9] demonstrations in perception* show, greatly influence the world in which we live. That world is in part, at least, what we make of it. Once our mold for world making is formed it most strongly resists change. The psychodelics allow us, for a little while, to divest ourselves of these acquired assumptions and to see the universe again with an innocent eye. In T. H. Huxley's[38a] words, we may, if we wish, "sit down in front of the facts like a child" or, as Thomas Traherne, a 17th-century English mystic puts it, "to unlearn the dirty devices of the world and become as it were a little child again."† Mystic and scientist have the same recipe for those who seek truth. Perhaps, if we can do this, we shall learn how to rebuild our world in another and better image, for our extraordinary technical virtuosity is forcing change on us whether we like it or not. Our old faults, however, persisting in our new edifice, are far more dangerous to us than they were in the old structure. The old world perishes and, unless we are to perish in its ruins, we must leave our old assumptions to die with it. "Let the dead bury their dead" tells us what we must do.

While we are learning, we may hope that dogmatic religion and authoritarian science will keep away from each other's throats. We need not put out the visionary's eyes because we do not share his vision. We need not shout down the voice of the mystic because we cannot hear it, or force our rationalizations on him for our own reassurance. Few of us can accept or understand the mind that emerges from these studies. Kant once said of Swedenborg, "Philosophy is often much embarrassed when she encounters certain facts she dare not doubt yet will not believe for fear of ridicule." Sixty years ago orthodox physicists *knew* that the atom was incompressible and indivisible. Only a few cranks doubted this, yet who believes in the billiard-ball atom now?

In a few years, I expect, the psychedelics that I have mentioned will seem as crude as our ways of using them. Yet even though many of them are gleanings from Stone Age peoples they can enlarge our experience greatly. Whether we employ these substances for good or ill, whether we use them with skill and deftness or with blundering ineptitude depends not a little on the courage, intelligence, and humanity of many of us who are working in the field today.

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* "... the principle that what we are aware of is not determined entirely by the nature of what is out there or by our sensory processes, but that the assumptions we bring from past experience, because they have generally proved reliable, are involved in every perception we have."[9]

† Also Francis Bacon, the father of modern scientific method, in *Novum Organum*, wrote "The entrance into the Kingdom of man, founded on the sciences, being not much other than the entrance into the Kingdom of Heaven, whereinto none may enter except as a little child."

Recently I was asked by a senior colleague if this area of investigation lies within the scope of science and, if it does not, should not religion, philosophy, or politics take the responsibility for it? But politics, philosophy, religion, and even art are dancing more and more to the tune of science and, as scientists, it is our responsibility to see that our tune does not become a death march, either physical or spiritual.

We cannot evade our responsibilities.

So far as I can judge, spontaneous experience of the kind we are discussing has always been infrequent, and the techniques for developing it are often faulty, uncertain, clumsy, objectionable, and even dangerous. Our increasingly excellent physical health, with the steady elimination of both acute and chronic infections; the tranquilizers that enable us to neutralize unusual chemo-electrical brain activity; our diet, rich in protein and, especially, B-complex vitamins whose antagonism to LSD I have already discussed; all of these combined with a society whose whole emphasis is on material possessions in a brightly lit and brilliantly colored synthetic world, will make spontaneous experiences of the sort I have mentioned ever fewer. As we grow healthier and healthier, every millimeter that we budge from an allotted norm will be checked.

I believe that the psychedelics provide a chance, perhaps only a slender one, for *homo faber*, the cunning, ruthless, foolhardy, pleasure-greedy toolmaker to merge into that other creature whose presence we have so rashly presumed, *homo sapiens*, the wise, the understanding, the compassionate, in whose fourfold vision art, politics, science, and religion are one. Surely we must seize that chance.

Summary

After indicating that there are a number of substances at present subsumed as psychotomimetic agents I have indicated that these are not yet clearly defined, and I have suggested that while mimicking psychoses is one aspect of these agents, it is not the only or even the most important one. I have discussed their great antiquity and have shown how they have attracted man since the dawn of history. Since many drugs produce changes in both body and mind, I consider that some working definition is required that will exclude anesthetics, hypnotics, alcohol, and the derivatives of morphine, atropine, and cocaine. I have suggested as a definition: "psychotomimetic agents are substances that produce changes in thought, perception, mood and sometimes posture, occurring alone or in concert, without causing either major disturbances of the autonomic nervous system or addictive craving, and although, with overdosage, disorientation, memory disturbance, stupor, and even narcosis may occur, these reactions are not characteristic."

This definition, of course, will be modified as knowledge grows.

I have discussed model psychoses induced by means of these agents and have indicated the existence of many gaps in our understanding. I believe that the

lack of such information has delayed the development of the sort of inquiry that has recently led to work with adrenochrome, adrenolutin, and bufotenin, mentioning some of the difficulties that beset those who work with the newer and truer psychotomimetics. I have suggested how model therapies modifying model psychoses and the study of means of aggravating or prolonging them provide useful information, and have touched on some of their uses in psychotherapy, emphasizing how much is still unknown.

I believe that there is a place for the use of these substances in the training of psychiatrists, psychologists, nurses, and others working with the mentally ill. I have linked these agents with recent work on the reduced and specialized environment by Hebb and Lilly, and I have discussed some psychological, social, and philosophical implications inherent in this inquiry, relating them to the newer work on perception.

In view of all these considerations, I have suggested that "psychotomimetic" is far too narrow a generic term, and I have suggested several that imply alterations in the normal mind. Among these proposed designations are "psychehormic," "psycherhexic," and "psychezymic," my own preference being "psychelytic," or "psychedelic"—mind-manifesting.

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THE DISTRIBUTION AND METABOLISM OF LYSERGIC ACID DIETHYLAMIDE

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Although lysergic acid diethylamide (LSD), the hallucinogenic agent, has been the subject of numerous investigations, little is known about its biologic fate. In recent studies Boyd *et al.*[1] and Stoll *et al.*[2] have examined the tissue distribution of C^{14}-labeled LSD in mice. Stoll and his co-workers[2] have shown that the C^{14}-labeled material measured in tissues included LSD, as well as transformation products of the drug. Lanz, Cerletti, and Rothlin[3] have also measured the tissue levels of LSD in mice by the use of a bioassay procedure based on the antagonism of LSD to serotonin-induced contraction of the uterus of the rat. These investigators, however, did not show that possible biologically active transformation products of LSD were excluded from the assay procedure.

The development of a specific and sensitive method for the estimation of LSD in biological materials has enabled us to study the tissue distribution, excretion, rate of biotransformation, and metabolism of the drug, as well as the subcellular processes involved in its transformation.

Methods and Materials

Estimation of LSD content. LSD was isolated from sodium chloride-saturated biological material at an alkaline *p*H by extraction into heptane. The LSD in the heptane extract was returned to dilute hydrochloric acid and its quantity was estimated spectrofluorometrically.

Procedure. Biological material (up to 5 ml.) was added to a 60-ml. glass-stoppered bottle that held 25 ml. of *n*-heptane containing 2 per cent isoamyl alcohol,§ 0.5 ml. of 1 N NaOH, and about 3 gm. of sodium chloride. The bottle was shaken for 30 minutes and then centrifuged. Twenty ml. of the heptane phase were transferred to a 40-ml. glass-stoppered centrifuge tube containing 3 ml. of 0.004 N HCl, and the tube was shaken for 10 minutes. An aliquot of the acid phase was transferred to a quartz cuvette, and the LSD content was determined by measuring its fluorescence in a Farrand or Bowman[4] spectrofluorophotometer at 445 mμ after activation at 325 mμ.¶ As little as 0.001 μg. per ml. of LSD could be determined by this procedure. Concentrations of the drug greater than 10 μg. may be measured spectrophotometrically at 310 mμ.

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§ All solvents were purified by successive washings with 1 N NaOH, 1 N HCl, and water.

¶ When LSD in dilute hydrochloric acid was exposed to light at 325 mμ for more than 2 seconds the intensity of the fluorescence of the compound gradually diminished. Upon removal from the light source for 5 minutes the intensity of fluorescence returned to its initial value.

TABLE 1

DISTRIBUTION OF LSD AND APPARENT LSD BETWEEN HEPTANE AND WATER AT VARIOUS *p*H VALUES*

*p*H	Authentic LSD	Apparent LSD from plasma
5.0	0.08	0.08
6.3	0.58	0.57
7.0	0.90	0.91
8.0	1.00	1.02
9.5	1.02	1.01

* The apparent LSD from the plasma of a cat that had received the drug intravenously was extracted into a heptane-isoamyl alcohol mixture as described under the heading *Methods and Materials*. Aliquots of this solution and a solution of authentic LSD in heptane (containing 2 per cent isoamyl alcohol) were shaken with one-quarter volumes of sodium-chloride-saturated buffers at various *p*H values. The fraction of the compound extracted was expressed as the ratio of the amount of compound in the organic phase to total compound.

Tissues were prepared for analysis by homogenization in a Waring blender with 5 volumes of water.

LSD added to biological material was recovered with adequate precision (95 ± 6 per cent).

Assay of specificity. In order to determine whether closely related transformation products of LSD were included in the measurement by the method described above, the specificity was assayed by means of the technique of comparative distribution ratios (Brodie, Udenfriend, and Baer).[5] To escape detection by this technique, metabolic products must simulate LSD to an improbable degree, in that they would have identical solubility characteristics in 2 solvents and similar dissociation constants. The distribution ratios of apparent LSD extracted from the plasma of a cat that had received the drug were compared with those of authentic LSD in a series of 2-phase systems consisting of heptane containing 2 per cent isoamyl alcohol and water at various *p*H values. The results shown in TABLE 1 indicate that the 2 compounds had the same solubility at various *p*H values and were presumably the same.

Preparation of tissue samples for enzyme studies. Preparation of all tissue samples was carried out at 0 to 3° C. Animals were stunned and exsanguinated, and the tissues were immediately removed and chilled. Tissue slices were prepared with a Stadie-Riggs* slicer. Liver preparations free of nuclei and mitochondria, but containing microsomes, were prepared by homogenizing the tissue with 4 volumes of isotonic KCl with a Potter-Elvehjem-type homogenizer* followed by centrifugation at 10,000 g for 15 minutes. Nuclei, mitochondria, microsomes, and soluble fractions were separated by fractional centrifugation (Schneider).[6]

Measurement of the enzymic conversion of LSD. In most studies on the enzymic conversion of LSD, 1 ml. of enzyme preparation, obtained from 200 mg. of tissue, was incubated in a 20 ml. beaker at 37°C. with 0.66 μmol of LSD, 0.2 μmol of triphosphopyridine nucleotide (TPN^+), 25 μmol of nicotinamide, 25 μmol of magnesium chloride, 500 μmol of potassium phosphate buffer (*p*H 7.9), and water to make a final volume of 5 ml. The mixture was shaken in a Dubnoff metabolic apparatus for 120 minutes in an atmosphere of

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* Manufactured by the Arthur H. Thomas Co., Phila., Pa.

TABLE 2
TISSUE DISTRIBUTION OF LSD IN THE CAT*

Tissue	LSD
	mg./kg.
Plasma	1.75
Cerebrospinal fluid	0.36
Brain	0.52
Liver	0.67
Kidney	0.53
Muscle	0.20
Heart	0.30
Lung	0.87
Spleen	0.38
Intestine	0.39
Fat	0.20
Bile	1.85

* The studies were made 90 minutes after the intravenous administration of 1 mg./kg. of LSD.

95 per cent O_2 to 5 per cent CO_2. At the end of the incubation period an aliquot of the reaction mixture was immediately taken for LSD analysis. Enzyme activity was expressed as μmol of LSD, which disappeared on incubation.

Materials. Triphosphopyridine nucleotide (TPN^+) of about 95 per cent purity and glucose-6-phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. Glucose-6-phosphate dehydrogenase was prepared by the procedure of Kornberg.[7] Lysergic acid diethylamide was obtained from the Sandoz Chemical Works, Hanover, N. J.

Results and Discussion

The distribution of LSD in tissues. The distribution of LSD was examined in representative tissues of a cat that had received 1 mg./kg. of the drug intravenously. Ninety minutes after administration of the drug, the animal was sacrificed and the tissues sampled immediately afterward (TABLE 2). LSD was found to be localized in the bile and plasma to a considerable extent, whereas only negligible amounts were present in the fat. The high concentration of LSD in the bile and the negligible amounts of the drug found in the feces (see below) would suggest that it was secreted into the intestines and then reabsorbed. In the order of decreasing magnitude, the concentration of LSD in the organ tissues was as follows: lung, liver, brain, intestines, spleen, cerebrospinal fluid, muscle, and fat. The presence of considerable amounts of the drug in the brain and cerebrospinal fluid indicates that the substance can pass the blood-brain barrier. No differences in the concentration of LSD in the cortex, midbrain, spinal cord, or hypothalamus were found. From the amount of LSD found in the brain of a cat after the administration of 1 mg./kg., it can be calculated that the drug exerts its psychological effect in man (given 1 μg. per kg.) at a concentration of 0.0005 μg. per gm. of brain tissue.

Since considerable amounts of LSD were found in the plasma, the extent to which the drug was bound to plasma proteins was determined by dialysis at

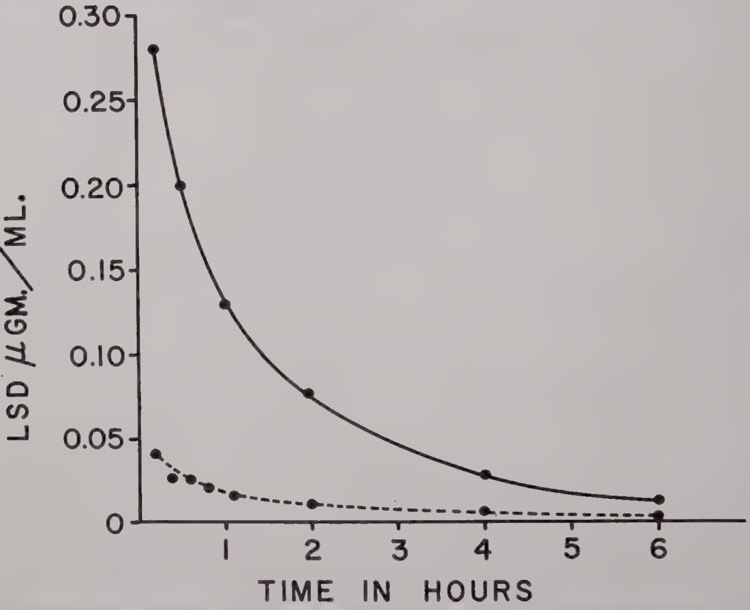

Figure 1. Plasma (solid line) and cerebrospinal fluid (dashed line) levels of LSD after the intravenous administration of 0.2 mg./kg. of the drug to a monkey.

37°C. for 18 hours against isotonic phosphate buffer at *p*H 7.4. Visking membranes* were used as dialysis bags. At plasma concentrations of 0.1 and 20 mg. per liter, 90 and 65 per cent of LSD, respectively, was found to be bound to the nondiffusable constituents of the plasma.

Relationship between plasma and cerebrospinal-fluid levels of LSD. Monkeys (*Macaca mulatta*) were given LSD (0.2 mg./kg.) intravenously. Blood and cerebrospinal fluid from the lumbar subarachnoid space were taken at various time intervals and assayed for LSD (figure 1). The rapid decline of the plasma level of the drug in the first hour was presumbaly due to a shift of LSD from plasma to tissues. After the first hour, the slower disappearance was a reflection of the rate of metabolism of the drug. The biologic half life of LSD in the monkey, that is, the time required for the plasma level to fall to half its value, was found to be about 100 minutes.

Maximum concentration of LSD in the cerebrospinal fluid was reached within 10 minutes, and then the amount declined slowly. Since the amount of drug present in the cerebrospinal fluid was about the same as that present in the unbound form in the plasma, it can be concluded that there was little hindrance to the passage of LSD across the blood-brain barrier.

Species differences in the rate of metabolism of LSD. Considerable species differences in the rate of metabolism of LSD were found (table 3). In mon-

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* Manufactured by The Visking Corporation, New York, N. Y.

TABLE 3

THE RATE OF METABOLISM OF LSD IN A NUMBER OF SPECIES

Species	Biologic half life
	Minutes
Monkey*	100
Cat*	130
Mouse†	7

* Three monkeys (*Macaca mulatta*) and 3 cats were given 0.2 mg./kg. LSD intravenously and the biologic half life was determined by the plasma-decay curve of the drug as described previously (Axelrod, Udenfriend, and Brodie[8]).

† Ten mice (N.I.H. stock) were given 2 mg./kg. LSD intraperitoneally. The mice were sacrificed 20 minutes after the administration of the drug, and the LSD concentration was determined after homogenizing the whole animal with 4 volumes of water in a Waring blender. The biologic half life of LSD was determined graphically by plotting the concentration of the compound at zero time and at 20 minutes on semilog paper.

keys and cats the drug was transformed at about the same rate, whereas in mice the LSD was metabolized very rapidly. In view of the speed with which LSD was transformed in mice, studies that require prolonged action by the drug in mice would presumably require its repeated administration.

Excretion of LSD. Three monkeys were given LSD (0.2 mg./kg.) intravenously, and then urine was collected for 24 hours, and feces were collected for 48 hours. Less than 1.0 per cent of the administered LSD was found in the urine or feces. Previous experiments had shown that the drug was not destroyed in stool suspensions after incubation for 18 hours at 37°C. These observations suggested that LSD underwent almost complete metabolic change in the body.

The site of metabolic transformation of LSD. Guinea pig liver, brain, kidney, spleen, and muscle (diaphragm) slices were incubated at 37°C. with 0.6 μmol of LSD in a Krebs-Ringer bicarbonate buffer in an atmosphere of 95 per cent O_2 to 5 per cent CO_2. At the end of 2 hours the incubation mixtures were homogenized and examined for the amount of LSD remaining. Liver tissue was the only tissue that metabolized the drug.

Requirements for in vitro *transformation of LSD.* The requirements for the enzymic transformation of LSD were determined in guinea pig liver microsomes and dialyzed soluble fractions. Maximal enzyme activity was obtained in the presence of TPN^+ and nicotinamide (TABLE 4). When DPN^+ was

TABLE 4

REQUIREMENTS FOR THE ENZYMIC TRANSFORMATION OF LSD*

	LSD metabolized
	μmol
Complete system	0.46
TPN^+ omitted	0.14
Nicotinamide omitted	0.10
DPN^+ substituted for TPN^+	0.23
Oxygen omitted	0.00

* The complete system contained microsomes and the dialyzed soluble fraction obtained from 200 mg. guinea pig liver, 0.2 μmol TPN^+, 50 μmol nicotinamide, 25 μmol $MgCl_2$, 500 μmol potassium phosphate buffer (*p*H 7.9) and 0.6 μmol LSD. The incubation time was 120 minutes at 37°C. in an atmosphere of 95 per cent O_2 to 5 per cent CO_2.

TABLE 5

INTRACELLULAR LOCALIZATION OF LSD-METABOLIZING ENZYME*

Intracellular fraction	LSD metabolized
	μmol
Whole homogenate	0.50
Nuclei	0.00
Mitochondria	0.00
Microsomes	0.00
Soluble fraction	0.00
Mitochondria + soluble fraction	0.05
Microsomes + soluble fraction	0.50

* Intracellular fractions obtained from 200 mg. of guinea pig liver were incubated at 37°C. for 120 minutes with 0.6 μmol LSD and cofactors described under the heading *Methods and Materials*.

substituted for TPN$^+$, activity was markedly diminished. Oxygen was required for the enzymic transformation of LSD, since incubation under anaerobic conditions resulted in negligible activity. Using potassium phosphate buffers, maximal enzyme activity occurred between *p*H 7.0 and 8.0.

Guinea pig liver was separated into nuclear, mitochondrial, microsomal, and soluble fractions, and each fraction was examined for its ability to metabolize LSD (TABLE 5). Little activity was found in any individual fraction but, when the microsomes were combined with the soluble fraction, enzyme activity was restored. From these observations it was concluded that factors present in both the microsomal and soluble fractions were necessary to transform LSD.

In previous studies on the *in vitro* metabolism of amphetamine (Axelrod[9, 10]), it was shown that the deamination of this compound was mediated by an enzyme system in liver microsomes that required TPNH and oxygen. The requirement for the microsomal and soluble fraction and TPN$^+$ suggested that the enzyme involved in the metabolism of LSD may also require TPNH, and that the TPN$^+$-dependent dehydrogenases in the soluble fraction served to generate the reduced cofactor. When a TPNH-generating enzyme system consisting of glucose-6-phosphate dehydrogenase and TPN$^+$ was added to the microsomes, these cofactors were as effective as the soluble fraction in metabolizing LSD (TABLE 6).

A microsomal enzyme system requiring TPNH and oxygen was initially described for the deamination of amphetamine (Axelrod[9]). Subsequently, it was found that TPNH-dependent enzyme systems can carry out a variety of reactions such as demethylation of sympathomimetic amines (Axelrod[11]), aminopyrine, and other alkylamines (LaDu *et al.*[12]), hydroxylation of aromatic compounds (Mitoma and Udenfriend[13]), side-chain oxidation of barbiturates (Cooper and Brodie[14]), and cleavage of aromatic ethers (Axelrod[15]). It seems unlikely that the TPNH-dependent enzyme systems serve only to metabolize foreign compounds. Although their normal substrates have not yet been found, these enzymes are probably engaged in carrying out normal metabolic functions as well as acting as "detoxifying" enzymes.

Evidence for the enzymic transformation of LSD to 2-oxyLSD. Possible metabolic pathways for the enzymic transformation of LSD may involve *N*-demethylation to yield norLSD and formaldehyde, hydrolysis to form lysergic acid,

TABLE 6

REQUIREMENT FOR TPNH*

Additions	LSD metabolized
	μmol
Microsomes + soluble fraction	0.53
Soluble fraction	0.00
Microsomes	0.04
Microsomes + 35 μmol of glucose-6-PO_4	0.04
Microsomes + 35 μmol of glucose-6-PO_4 + 1 mg. of glucose-6-PO_4 dehydrogenase†	0.48

* The microsomes and the soluble fraction were prepared by fractional centrifugation of a homogenate of 200 mg. of guinea pig liver in 5 volumes of isotonic KCl. The reaction mixtures contained 500 μmol of potassium phosphate buffer (*p*H 7.9), 25 μmol of $MgCl_2$, 1 μmol TPN^+, 25 μmol of nicotinamide, 0.66 μmol of LSD, and water to make a final volume of 5.0 ml. Incubation time 120 minutes at 37°C. in an atmosphere of 95 per cent O_2 to 5 per cent CO_2.

† 2.4 Kornberg units per mg. of protein.[7]

or oxidation of the indole ring. The extent of the enzymic demethylation of *N*-methyl-substituted compounds can be determined by measuring the formaldehyde formed (Axelrod[16]). When 5 μmol LSD were incubated with guinea pig microsomes, soluble fraction, cofactors described under the heading *Methods and Materials*, and 100 μmol of semicarbazide to trap formaldehyde, no formaldehyde was found.

The formation of lysergic acid or oxidized LSD was examined after incubating 30 μmol of LSD with microsomes and a soluble fraction obtained from 20 gm. of guinea pig liver, 10 μmol of TPN^+, 500 μmol of nicotinamide, 250 μmol of $MgCl_2$, and 50 ml. of *p*H 7.9 phosphate buffer (0.2M) at 37°C. After 3 hours of incubation, the reaction mixture was saturated with NaCl, made alkaline, and the residual LSD was removed by shaking with 3 portions of a heptane-2 per cent isoamyl-alcohol mixture. An aliquot of the aqueous phase was adjusted to *p*H 6.0 and shaken with 4 volumes of isoamyl alcohol. The isoamyl-alcohol extract was then shaken with one-fifth volume of 0.1 N NaOH. The alkaline extract showed negligible absorption at 310 mμ. Lysergic acid has an absorption peak at 310 mμ and can be quantitatively extracted under the conditions described above.

Another aliquot of the reaction mixture that was washed free of residual LSD was adjusted to *p*H 9.0 and then extracted with 5 volumes of *n*-butanol. Five volumes of *n*-heptane were added to an aliquot of the butanol extract and the mixture was shaken with one-tenth volume of 0.1 N HCl. An aliquot of the acid extract gave a Folin-Ciocalteu[17] reaction, indicating the presence of a reducing indole. Unlike LSD, the metabolite gave no reaction with Hopkins-Cole or Van Urk's[18] dimethylaminobenzaldehyde reagents. These observations suggested that substitution had occurred on position 2 of the indole ring. Since alkaline hydrolysis is known to cleave oxindoles at the amide linkage to a salt of an amino acid containing a diazotizable amino group (Witkop[19]), the LSD metabolite was heated at 100°C. in 2 N NaOH for 5 minutes, cooled, and subsequently treated with sodium nitrite in an acid solution. An intense wine color appeared when the diazotization mixture was added to an alkaline solution of β-naphthol. These observations indicated that LSD was converted

Table 7

Balance Study on the Enzymic Conversion* of LSD to 2-oxyLSD

Experiment No.	LSD metabolized	2-oxyLSD formed
	μmol	*μmol*
1	0.61	0.52
2	0.64	0.62
3	0.68	0.64

* Guinea pig liver microsomes and the soluble fraction obtained from 200 mg. of liver were incubated at 37°C. for 120 minutes with 0.7 μmol LSD and cofactors described under the heading *Methods and Materials*. At the end of the incubation period the reaction mixture was assayed for LSD and 2-oxyLSD.

to 2-oxyLSD by the TPNH-dependent microsomal enzyme system. Enzymatically formed 2-oxyLSD and synthetic 2-oxy LSD (synthesized by K. Freter, J. Axelrod, and B. Witkop, of the National Institutes of Health) were found to be the same with respect to their ultraviolet-absorption and ultraviolet-fluorescence spectra, distribution coefficients in various organic solvents, R_f values on paper chromatograms and chemical tests. Preliminary studies have shown that ergotamine may undergo a similar transformation.

A balance study on the enzymic conversion of LSD to 2-oxyLSD was made by measuring the disappearance of LSD and the formation of 2-oxyLSD in the microsomal preparations. The formation of 2-oxyLSD was determined in the following manner:

Two ml. of the incubated reaction mixture were made alkaline, saturated with sodium chloride, and the unreacted LSD was removed by extraction with 2 portions of *n*-heptane containing 2 per cent of isoamyl alcohol. The aqueous residue was adjusted to *p*H 9, and the 2-oxyLSD was extracted into 15 ml. of *n*-butanol. Ten ml. of the butanol extract were transferred to a 40 ml. glass-stoppered centrifuge tube containing 20 ml. of *n*-heptane and 1.5 ml. of 0.1 N HCl and the contents of the tube were shaken for 10 minutes. To 1 ml. of the acid extract, 0.1 ml. of Folin-Ciocalteu reagent[17] and 0.2 ml. Na_2CO_3 (20 per cent solution) were added. The solution was heated for 1 minute in a boiling water bath, cooled, and its optical density at 630 mμ was determined in a Beckman* spectrophotometer adapted for small volumes. A known amount of 2-oxyLSD,† which was incubated and extracted in the same manner as described above, served as a standard. From the results shown in TABLE 7, it appeared that approximately 1 mol of 2-oxyLSD was formed for each mol of LSD that was metabolized.

Activity of 2-oxyLSD in the central nervous system. Previous studies have shown that LSD blocks synaptic transmission in the lateral geniculate nucleus and reduces spontaneous cortical activity (Evarts *et al.*[20]). An intracarotid administration of 0.4 mg./kg. of 2-oxyLSD in cats that had received Nembutal did not alter the postsynaptic response to optic-nerve shock, whereas 0.03 mg./kg. of LSD resulted in an 80 per cent decrease in the amplitude of the postsynaptic response. We also found that 2-oxyLSD was without effect on the spontaneous cortical activity recorded during barbiturate anesthesia. The

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* Manufactured by the Pyrocell Manufacturing Co., New York, N. Y.

† On this occasion, 2-oxyLSD used as a standard was previously isolated from a microsomal preparation incubated with LSD. The concentration of the standard solution of 2-oxyLSD was determined by comparing its optical density at 630 mμ with that of LSD after treatment with Folin-Ciocalteu reagent.[17] It was assumed that both LSD and 2-oxyLSD have the same molecular extinction at 630 mμ after treatment with this reagent.

TABLE 8

INHIBITION OF LSD METABOLISM *IN VITRO**

Additions	Per cent inhibition
Serotonin 1×10^{-3}M	40
Reserpine 1×10^{-3}M	20
Chlorpromazine 1×10^{-4}M	70
SKF 525 1×10^{-4}M	90

* Guinea pig microsomes and the soluble fraction were incubated at 37°C. for 60 minutes with 6×10^{-5} M LSD, inhibitors and cofactors as described under the heading *Methods and Materials.*

oral administration of 300 μg. of 2-oxyLSD did not produce any psychological effects in a human subject who responded to 30 μg. of LSD. It may be concluded on the basis of this evidence that 2-oxyLSD does not possess LSD-like activity in the central nervous system.

Inhibitors of the metabolism of LSD. Since other investigators have demonstrated biologic interactions between LSD, serotonin, and tranquilizing agents (Gaddum,[21] Wooley,[22] and Shore, Silver, and Brodie[23]), we examined the effect of serotonin, reserpine, and chlorpromazine on the metabolism of LSD by the microsomal preparation (TABLE 8). It was found that chlorpromazine markedly inhibited the enzymic oxidation of LSD at relatively low concentrations (1×10^{-4}M), while serotonin and reserpine inhibited at higher concentrations (1×10^{-3}M). Since chlorpromazine exerted marked inhibitory action on the metabolism of LSD *in vitro*, the effect of this compound on the *in vivo* metabolism of LSD was examined in mice. The biologic half life of LSD (2 mg./kg. administered intraperitoneally) was 7 minutes. When chlorpromazine (100 mg./kg.) was administered intraperitoneally 5 minutes prior to the injection of LSD, the biologic half life was increased to 15 minutes.

The inhibitory action of SKF 525 (β-diethylaminoethyl diphenylpropyl acetate) on the enzymic oxidation of LSD was also examined. This compound has been shown to inhibit many TPNH-dependent microsomal enzymes both *in vivo* (Axelrod, Reichenthal, and Brodie[24]) and *in vitro* (Cooper, Axelrod, and Brodie[25]). SKF 525 at a concentration of 1×10^{-4}M almost completely blocked the enzymic transformation of LSD (TABLE 8).

Summary

(1) A specific and sensitive method for the estimation of LSD in biological material is described.

(2) After administration of LSD to the cat, the drug is found in all tissues in the following order of decreasing concentrations: bile, plasma, lung, liver, kidney, brain, intestines, spleen, cerebrospinal fluid, muscle, and fat. The drug is extensively bound to plasma proteins, and there is no hindrance in the passage of the drug across the blood-brain barrier.

(3) LSD is almost completely metabolized in the body, only negligible amounts of the drug being excreted in the urine or the stools. The liver is the major site of metabolism of the drug.

(4) There are considerable species differences in the rate of biotransformation of LSD.

(5) LSD is transformed to 2-oxyLSD by an enzyme system present in liver microsomes that requires oxygen and a reduced-triphosphopyridine nucleotide generating system. The new compound, 2-oxyLSD, does not possess LSD-like activity in the central nervous system.

(6) Chlorpromazine (1×10^{-4}M) and SKF 525 (1×10^{-4}M) markedly inhibit the *in vitro* metabolism of LSD, while serotonin (1×10^{-3}) and reserpine (1×10^{-3}M) inhibit the *in vitro* metabolism moderately.

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A REVIEW OF BIOCHEMICAL CHANGES INDUCED *IN VIVO* BY LYSERGIC ACID DIETHYLAMIDE AND SIMILAR DRUGS

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Studies of the physiological and biochemical factors associated with the so-called functional psychoses such as schizophrenia are surrounded by special difficulties. Perhaps the foremost of these is the fact that while what appears to be neurotic behavior can be produced in experimental animals, psychoses per se have not been satisfactorily demonstrated, and investigations have been limited, for the most part, to human beings. Most psychotics available for study are patients in state hospitals where, except for a few institutions that are notable exceptions to the rule, custodial care is routinized, and experiments with patients are discouraged. The patients in state hospitals are wards of the respective states, and the massive weight of bureaucracy is not conducive to the fluidity of attack and the imaginative approach necessary for effective research. Because of social attitudes toward mental patients and the isolation of much of psychiatry from the main stream of the biological and medical sciences, funds for physiological and biochemical research in the various aspects of mental disorders have been small. These limitations have resulted in meager advances in knowledge of neurophysiological and biochemical events underlying the disturbances of higher mental processes, and basic research in this field has been to date of little help to the practicing psychiatrist.

Partly as a result of these limitations and partly as a consequence of the impact of the psychodynamic schools of thought with primary interest in psychotherapy, the basic investigations of the biology of schizophrenia have received scant encouragement either from psychiatrists or from the general public. Relatively few biochemists and physiologists have been attracted to the field in the past 2 or 3 decades. Quite recently this situation has changed as a result of the reawakened interest in psychopharmacology brought about by the psychotomimetic and tranquilizing drugs. This lack of interest in the biochemical approach has existed despite notable successes of various empirical advances in physiological therapies for mental patients, such as electroshock, metrazol, and insulin treatments, leukotomy and, more recently, the tranquilizing drugs. Very impressive, too, have been studies leading to the virtual eradication of 2 forms of major psychoses that were prominent in the past. Advances in syphilology have virtually eliminated general paresis, a psychosis often difficult to distinguish from schizophrenia by purely psychiatric criteria. The psychosis of pellagra, so prevalent in the southern part of the United States a generation ago, has also been relieved by correcting a chemical lesion due to a coenzyme deficiency. Both of these disorders were once regarded as "functional" in nature before their physical bases were understood. Today schizophrenia is regarded as a "functional psychosis," in contrast to an "organic psychosis," implying to many that it involves no molecular lesion, but is a result of psychological experiences subject to correction by psychotherapy if such treatment is practiced long enough and skillfully enough on a given patient.

In contrast to this attitude is the view that a behaving disembodied psyche is a meaningless concept, and that all behavior, both "normal" and "abnormal," is a result of the organized activities of a brain functioning in its internal environment in which cellular dynamics determined by enzyme constellations and a host of chemical determinants are basic to the nature of behavior. The personal developmental history of an individual in relation to his environment can, by conditioning, modify within limits various aspects of the function of the brain, but why some persons develop schizophrenia in the face of life's stresses and others do not is the prime unanswered question. The role of genetic factors predisposing towards schizophrenia, particularly as elucidated by Kallmann,[1] is of interest since the genes are the determinants of the enzymes that regulate and coordinate cellular function.

The paucity of basic knowledge as of 1956 is due to limitations of our current biochemical and physiological techniques and to the very brief time and low intensity with which such techniques have been applied to studies of the mentally ill. Mind and body are 2 aspects of the same behaving person. Psychotherapy may be helpful to some clearly organic disease states, as well as in the so-called functional disorders. Thus physical cripples and patients with gross brain damage may be helped by psychotherapy. The fact that some psychological tests differentiate macroscopic brain-damage cases from schizophrenics merely suggests to some of us that such tests differentiate between different kinds of brain lesions, not that the schizophrenic is free from molecular lesions of a different nature from those encountered in the so-called organic psychoses. To refer to schizophrenia as a functional disorder is merely to say that its molecular determinants are unknown.

Basic research aimed at understanding, rather than at applied results, has been difficult in most fields of endeavor. It does, however, furnish the capital that ultimately pays the dividends both in industry and in bedside medicine. I am constrained in this connection to quote Sigmund Freud, who once wrote in a letter to a young colleague, "I should advise you to try to find out what is happening. When you have done that, the therapy will take care of itself."

Psychotomimetic drugs are tools capable of producing brief and controllable experimental psychoses in normal persons, and they thus open new channels for the investigator. I have been asked to review *in vivo* biochemical studies involving lysergic acid diethylamide (LSD) and related drugs. Other investigators whose work appears in this monograph will discuss *in vitro* biochemical studies and various psychological and neurophysiological aspects of the action of these drugs. This review will therefore not attempt to cover these fields. It will also be concerned primarily with the effects of these agents in man. I am much indebted to the Sandoz Chemical Works, Inc., for furnishing abstracts of the literature on the subject, together with a collection of reprints that have been of great assistance in writing this review.

TABLE 1
PERCENTAGES OF THEORETICALLY EQUAL DISTRIBUTION OF COUNTS

Hypothalamus	16	Cortex	31
Cerebellum	26	Thalamus	28
Brainstem	17	Liver	135

Some Biochemical Effects of LSD

In this paper we shall first review some relatively unrelated biochemical studies, and then we shall consider these and other investigations in the light of certain hypotheses concerning biochemical determinants of the psychoses.

Hoagland[2] has reported studies using C^{14}-labeled LSD administered intraperitoneally to rats. Seven rats were injected with 100 μg. of LSD* labeled in the stable diethylamide position and of a specific activity of 8 mc. per mg. The animals were sacrificed 30 minutes after the injection. The brains of the rats were removed and dissected into cerebellum, brainstem, cerebral cortex, thalamus, and hypothalamus. Each of these areas was pooled from all of the rats. Livers were also removed and pooled. Counts were then made, using a gasometric technique, on each pooled sample after converting the carbon of the samples to CO_2. TABLE 1 shows the distribution of counts 30 minutes after injection of the labeled LSD.

Boyd *et al.*[3] showed that from 48 to 72 per cent of the count of a dose of radioactive LSD administered intraperitoneally to rats was found in their gastrointestinal tracts an hour after injection. An appreciable level of counts was present in the liver at this time. In a later paper, Lanz, Cerletti, and Rothlin[4] studied the LSD content of the blood, liver, brain, and skeletal muscle of mice given 35 mg./kg. of LSD intravenously. These investigators did this by testing the antagonism to serotonin of tissue extracts of the uterus of the rat. This reaction is fairly specific under the experimental conditions employed. LSD disappeared from the blood relatively rapidly and was found in various organs, including the brain, liver, and skeletal muscle. The LSD content of liver was particularly high, and the ratio of tissue LSD to blood LSD was constant. The LSD content of the brain decreased more rapidly than did that of the liver, and it did so independently of the blood level. At 37 minutes after injection, one half of the injected LSD had disappeared from the blood. Axelrod, in this monograph, reports an extensive study of the distribution and metabolism of LSD in several animals.

Forrer and Goldner[5] reported a study of 6 patients who were given as much as 6 μg. of LSD per kg. These investigators found that no change occurred in nonprotein nitrogen levels, and they also found that cephalin-cholesterol flocculation tests were normal. Fischer, Georgi, and Weber,[6] in studies of the effects of LSD (in doses of 130 μg.) and mescaline (in doses of 0.5 gm.) report that LSD causes a less intensive and more transient disturbance in liver function in man than does mescaline and they also state that Quick's hippuric-acid test for liver function is negative after dosage with LSD, but that it is positive after dosage with mescaline. This test is also positive in many cases of acute schizophrenia. According to these workers, the cinnamic-acid test of Snapper-Saltzman does, however, reveal some disturbances of liver function after the administration of LSD. This test is more sensitive than the Quick test.

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* The LSD used in this experiment was supplied by the Sandoz Chemical Works, Inc., Hanover, N. J.

Mayer-Gross, McAdam, and Walker[7] have suggested that because LSD acts in such small amounts to produce psychoses in man (as little as 0.5 μg./kg.) that it may function as an antienzyme in the brain, acting upon a special receptor substance. These workers have investigated the effect of LSD on carbohydrate metabolism *in vivo*, and they report that LSD produces an increase in blood hexose monophosphate due to a partial block in the breakdown of this metabolite. These investigators suggest that changes in carbohydrate metabolism may be responsible in part for the psychological symptoms caused by dosage with LSD, although the evidence supporting this suggestion is not convincing. In a later paper[8] the same authors found, in a study of 13 fasting schizophrenics given 80 μg. of LSD orally, that minimal psychological effects were produced, and that these effects were accompanied by an increase in blood hexose monophosphate. In control studies using water as a placebo, a decrease in blood hexose monophosphate was observed in 9 patients. Such a decrease, of course, would be expected in fasting subjects. Mescaline (3,4,5-trimethoxyphenylethylamine) given to 9 normal subjects produced psychological effects similar to those caused by LSD, but it caused no changes in blood carbohydrate. Studies in guinea pig brain tissue in the Warburg apparatus showed that the administration of LSD decreased the breakdown of hexose monophosphate in brain and liver tissue. These *in vitro* results could, however, not be confirmed (see James A. Bain, elsewhere in this monograph). In another study these authors[9] reported that in 24 persons LSD blocked glycogen decomposition at the phase of hexose monophosphate, thus preventing further cleavage to the end products, pyruvic acid and lactic acid. If the accumulation of hexose monophosphate in the blood is responsible for psychic symptoms by blocking energy release, the intravenous administration of glucose, which is broken down directly through the stage of hexose-6-phosphate, might abolish the toxic symptoms of LSD. They report that this seemed to be the case when blood-sugar values were elevated to 200 mg. per cent, but they further report that the number of experiments was too small to be conclusive.

Liddell and Weyl-Malherbe[10] have studied the clinical and biochemical effects of LSD (40 to 60 μg., administered intravenously) and Methedrine (methamphetamine) (40 to 50 mg., given intravenously) on patients suffering from various mental disorders. These authors have reported that after administration of both drugs the plasma epinephrine rose in the first hour, then dropped below the starting level in the second hour, finally rising again to a point well above the initial level. The effects of LSD on the blood-sugar concentration were hardly significant, although Methedrine caused some increase in blood-sugar levels. The methods of determining blood epinephrine and norepinephrine used by these authors, however, have not been successful in the hands of other investigators, so these findings remain unconfirmed.

Sloane and Doust[11] studied effects of LSD in 11 healthy control subjects, 12 depressive patients, and 7 schizophrenics. Spectroscopic oximetry following the administration of LSD was used as an estimate of the capillary change through oxygenation of blood during a standardized apnoeic stress before and during the time of action of the LSD. The results indicated an increased autonomic lability in the normal controls and in the depressives under LSD, but they evidenced no change in the schizophrenics, thus showing relative unresponsiveness of the capillary circulation to LSD in the schizophrenic group.

Elmadjian, Hope, and Lamson[12] studied 6 chronic schizophrenic patients and 9 patients with involutional melancholia and manic-depressive psychosis in the depressed phase, and 1 manic-depressive in the manic phase. From 10:00 A.M. to 2:00 P.M. a control urine specimen was collected and, at 2:00 P.M. the patients were given LSD orally in doses of from 100 to 300 μg. for the chronic schizophrenics and 150 μg. for the other patients. Samples of urine were again collected from 2:00 P.M. to 10:00 P.M. All urine samples were analyzed for epinephrine and norepinephrine. Mental status examinations and Malamud rating-scale determinations were made on the patients before and during the time of action of the LSD.

None of the schizophrenics showed observable psychiatric responses, mental status changes, or autonomic responses to the LSD. Larger doses (as much as 300 μg.) have been found necessary by these workers to get responses from most chronic schizophrenics. The 10 other subjects all showed positive responses to the LSD. They displayed changes in space-time perception, as well as hallucinations, delusions, disorientation, tenseness, and anxiety. Autonomic changes such as palpitation, sweating, rapid respiration, and blushing were observed and, in many cases, slurring of speech and ataxia. Most interesting of all was the finding that the chronic schizophrenics, all of whom failed to respond to the LSD, also showed no significant changes in the urinary excretion of epinephrine or norepinephrine. All of the other patients showed significant increases in urinary epinephrine and/or norepinephrine following the administration of LSD.

Since LSD is an ergot alkaloid it might be expected to inhibit responses to epinephrine, as is the case with other substances of this class. Graham and Khalidi[13] have found that LSD does inhibit, to varying degrees, the stimulating actions of epinephrine and norepinephrine on various smooth-muscle preparations and on the isolated perfused rabbit heart. LSD increases the pulse rate and blood pressure in man, and it sometimes increases the respiratory rate.[13, 14] These effects are much more pronounced in normal subjects than they are in schizophrenics.[13]

Buscaino and Frongia[15] have reported hypoglycemia and hyperbilirubinemia in dogs chronically poisoned with very large doses of LSD administered intramuscularly over a period of many days. Histochemical reactions in the brain were modified, and the liver and kidneys exhibited degenerative changes in the parenchyma. These authors suggest that their findings of pathological effects in the liver may be relevant to the frequent occurrence of liver impairment that has been reported as the result of tests on schizophrenic patients. Anderson and Rawnsley[16] have found that oral glucose tolerance is somewhat reduced in subjects under the influence of LSD. These authors observed this effect in 6 subjects during 9 administrations of LSD in amounts sufficient to produce experimental psychoses. The authors do not attempt to evaluate the significance of this finding in view of such presumed secondary effects of LSD as the mobilization of epinephrine through affective changes that in themselves would influence carbohydrate levels. They note in this connection that Forrer and Goldner[5] observed a slight rise in the blood sugar of fasting subjects beginning in the second and third hour after the administration of LSD. Mayer-Gross, McAdam, and Walker,[7, 8] also noticed a slight rise in the glucose levels of fasting patients after the administration of LSD. While different mechanisms are probaby involved, it is worth noting that about two thirds of a large group of schizophrenic patients studied by Freeman[17] displayed reduced sugar-tolerance curves as measured by the Exton-Rose oral-glucose procedure.

As has been reported in other cases schizophrenic patients as a group are less responsive to LSD than are either normal controls or other types of patients. A greater resistance of mental patients, including schizophrenics, to LSD in contrast to that of normal controls has been reported by Condrau.[18] Belsanti[19] reports that schizophrenics made poorer responses to LSD than did oligophrenics. J. Elkes, in a personal communication, has found schizophrenics to be relatively unresponsive to LSD, as compared to normal controls. We have already mentioned that Sloane and Doust[11] found, by spectroscopic oximetry, an increased autonomic lability following the administration of LSD in normal controls and depressive patients, but not in schizophrenics. Savage[20] found that 10 μg. of LSD produced effects in normal subjects, but that 100 μg. were necessary to produce comparable effects in schizophrenic patients. Cline and Freeman[21] in an investigation of 40 psychotics found that chronic schizophrenics, in contrast to normal controls, required much more LSD to produce psychological effects. We have noted that Elmadjian, Hope, and Lamson[12] found that schizophrenics, in contrast to depressives, are unresponsive to LSD both psychiatrically and in terms of autonomic responses. In contrast to its effect on depressives, LSD does not enhance the output of epinephrine and norepinephrine. Graham and Khalidi[13] found changes in blood pressure and pulse rate following the administration of LSD to be much more pronounced in normal controls than in schizophrenics.

In repeated experiments testing the effects of LSD on the same individual, care must be taken to avoid adaptation to the drug. Isbell *et al.*[22] found that tolerance to the effects of LSD developed in 7 days in psychiatric patients when the drug was given daily, but that it disappeared 3 days after discontinuance of the drug.

Some Biochemical Comparisons of Schizophrenia with LSD-Induced Psychoses in Normal Controls

Hoagland, Rinkel, and Hyde[23] have examined inorganic-phosphate excretion and some urinary indices of adrenocortical responsiveness in the course of 21 experiments on 12 normal men before and after taking LSD. These findings were compared further to extensive data of phosphate excretion and adrenocortical responsiveness obtained from large groups of drug-free schizophrenic men and normal men. These latter data were obtained over a period of years by investigators at the Worcester Foundation for Experimental Biology and the Worcester State Hospital, Worcester, Mass. With respect to comparisons of schizophrenics with normal persons, neither group receiving drugs, it was found that the schizophrenics displayed a urinary inorganic-phosphate excretion at rest of only about one half that of normal control persons within the same age range. In response to the administration of 25 mg. of adrenocorticotropic hormone (ACTH), the schizophrenics showed a somewhat lower increase in 17-ketosteroids, sodium, potassium, and uric acid output than do the normal controls. The phosphate excretion, on the other hand, while abnormally low before the administration of ACTH, showed a remarkable increase in output after its injection. This is not seen in the normal controls. Similar sharp rises in inorganic-phosphate excretion were seen in the schizophrenic group, but not in the normal controls, following the administration of adrenocortical extract or exposure to experimental stresses.

After taking LSD (0.5 to 1.0 μg./kg.) the normal men studied by Hoagland, Rinkel, and Hyde[23] showed a marked reduction in urinary phosphate excretion in samples collected over a 6-hour period after medication as compared with their control values before medication for samples covering the same time of day. The injection of ACTH, during the period of action of LSD, enhanced the phosphate excretion. Thus the behavior of phosphates both at rest and under the impact of adrenocorticoids in the LSD-treated normal person is similar to that found in schizophrenics without LSD. The data of this study also indicated that LSD appears to stimulate the pituitary adrenal axis and leaves the adrenal somewhat unresponsive to ACTH as measured by 17-ketosteroids, urinary sodium, and uric acid. The adrenals of schizophrenic patients (without the drug) as measured by changes in percentage in these particular urinary variables are also subnormally responsive to ACTH.

Bergen and Beisaw[24] in our laboratory have found that 50-μg. doses of LSD given to 400-gm. guinea pigs reduce phosphate excretion. Larger and smaller doses are not as effective. ACTH injected during the presumed period of action of LSD produces in guinea pigs, as in man, a rebound of phosphate excretion. Recent experiments indicate, however, that a rebound of phosphate excretion above normal levels occurs about 8 to 16 hours after injection of LSD in the guinea pig without any ACTH administration. If ACTH and LSD are given together at the start of the experiment, however, ACTH prevents, in guinea pigs, the fall in phosphate excretion due to the LSD. It is possible that the reduced phosphate excretion may be a result of oliguria that has been demonstrated[13] to follow the administration of LSD. Schizophrenic patients who have not received LSD display, on the other hand, diuresis when compared to normal persons and, at the same time, they show reduced phosphate excretion.[25] Respiratory movements may be increased slightly by LSD,[13, 14] and enhanced respiration has been associated with decreased phosphate excretion accompanying shifts in acid-base equilibrium in the blood.[26] F. Elmadjian, in a personal communication, has suggested that the low phosphate-excretion values that accompany treatment with LSD may be a result of small increases in respiration. This would not account, however, for the low phosphate-excretion data of schizophrenics as compared to those of normal controls, since schizophrenics in general have low volumes. The parallel between phosphate excretion in schizophrenics and that in normal persons before and after the administration of ACTH and the phosphate response of normal men before and after the administration of LSD, and to ACTH during the period of action of LSD, has led us to postulate that schizophrenic patients may produce an abnormal metabolite that acts after the manner of LSD, not only in producing psychotic behavior but also in affecting phosphate metabolism and adrenal responsiveness as measured by the urinary indices.

In this connection it is of interest that abnormalities of phosphate turnover in schizophrenic patients have been reported by Örström[27] and by Örström and Squag.[28] Boszormenyo-Nagy and Gerty[29] have also found abnormalities of phosphorylation by the erythrocytes of schizophrenics (other than schizophrenics of the simple type) in contrast to normal controls, manic depressives, diabetics, and patients with hyperthyroid conditions. As mentioned earlier, work from the laboratory of Mayer-Gross[7-9] indicates that LSD facilitates the accumulation of hexose monophosphate in blood. Our own data are consistent with the view that LSD and some endogenous metabolite that acts in a similar manner in schizophrenics either facilitates the conjugation of phosphates with organic substances or decreases the phosphate turnover rates. The role of adrenal steroids, as seen in the enhanced output of urinary phosphates following the administration of ACTH, appears to be either to release the conjugated phosphate, speed the turnover rate, or both. That adrenal steroids modify phosphorylating mechanisms by affecting several phosphorylating enzyme systems has been demonstrated.[30]

Hoagland, Rinkel, and Hyde[23] have suggested that the high threshold of responsiveness of schizophrenics to LSD that has been discussed above may result from their production of a possible endogenous metabolite that competes with LSD for enzyme receptors, thus reducing the effectiveness of LSD in these patients. Hoagland[31] has discussed the role of possible derangements of enzyme kinetics in the psychoses. In this connection Kallmann's extensive studies[1] of genetic factors in schizophrenia, particularly those of homozygous and heterozygous twins, clearly demonstrate that there are predisposing genetic factors for schizophrenia. Since the genes are the templates that form the enzymes, some abnormalities of enzyme production are thus implicated in these patients.

Intermediary metabolic derangements would appear to constitute the basic chemical lesion in schizophrenia. While there is nothing new in this general idea, studies of experimental psychoses in recent years have focused especial attention on the catechol amines and their derivatives and on serotonin or 5-hydroxytryptamine formed from tryptophan and metabolized through various steps that may include bufotenin primarily to 5-hydroxyindoleacetic acid.[31a] The chemical steps involved in the formation and breakdown of these compounds are considered more appropriately in Udenfriend's contribution to this monograph. Suffice it to say here that compounds in these groups are usually characterized by indole or indolelike rings. FIGURE 1 shows the structures of a number of compounds of interest in this connection.

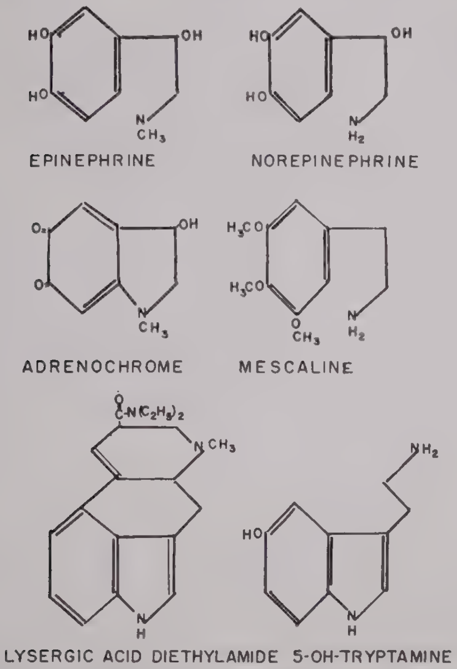

FIGURE 1. Illustration of the similarities of 3 psychotomimetic substances: adrenochrome, mescaline, and LSD. Epinephrine and norepinephrine have been suggested as possible precursors, in some persons, of abnormal amounts of endogenous metabolites such as adrenochrome that may act to produce clinical psychoses. The structural similarities of LSD and 5-hydroxytryptamine (serotonin) is shown also (figure by A. Slocombe).

Considerations of Epinephrine Derivatives as Agents in Clinical Psychoses

Quastel and Wheatley[32] in 1933 pointed out that a number of amines, including mescaline (3,4,5-trimethoxyphenylethylamine), strongly inhibit glucose, lactate, and pyruvate oxidation by the brain enzymes. Mann and Quastel[33] in 1940 showed that a number of amines including tyramine, indolethylamine (serotonin) and epinephrine all compete with each other for amine oxidase in the brain. These authors considered that aberrant amine metabolism may be involved in mental disease. Osmond and Smythies[34] in 1952 pointed out the structural similarities of the hallucinogen, mescaline, to epinephrine, and they suggested that disturbances in epinephrine metabolism might produce a toxic catechol amine capable of causing psychosis. Hoffer, Osmond, and Smythies[35] reported, on the basis of 8 experiments, that adrenochrome, an oxidation product of epinephrine that may also be formed in the body, produced psychoticlike disturbances on injection, and they suggested that it may be a primary causal factor in schizophrenia. More recently, H. Hoffer, in a personal communication, has also reported adrenolutin, another epinephrine-oxidation product, to be psychotomimetic. It is of interest and relevant to the findings of Mayer-Gross, McAdam, and Walker[7-9] of the accumulation of hexose monophosphate following the administration of LSD and also other findings, cited above, concerning the abnormalities of phosphorylation in schizophrenics and in normal men and guinea pigs following the administration of LSD, that Meyerhof and Randall[36] have reported that glucose and hexose monophosphate degradations in the presence of brain enzymes *in vitro* are markedly inhibited by adrenochrome. Thus both adrenochrome and LSD modify phosphorylations.

There are a number of derivatives of epinephrine and of norepinephrine that, if isolated, may yield results of interest. Thus the pharmacological properties of adrenoxin as described by Heirman[37] led Rinkel[23] to suggest this substance, another oxidation product of epinephrine, as one of possible psychotomimetic interest. Since, in contrast to epinephrine, norepinephrine has been found to be present in some quantity in the brain, especially in the hypothalamus,[38] derivatives of this substance eventually may turn out to be of more interest than those of epinephrine as possible endogenous agents responsible for clinical psychoses.

Serotonin (5-hydroxytryptamine), LSD, and Reserpine in Relation to Mechanisms of the Psychoses

Some of the most interesting and recent developments in the field of experimental psychoses are subsumed under the above heading. A great part of this monograph deals with reviews by others of this material and with the presentation of original investigations in this field. We shall therefore consider studies of the interrelations of the actions of serotonin, LSD, and reserpine only briefly.

The reader is especially referred to a paper by Woolley and Shaw[39] for a stimulating discussion of the possible role of serotonin as a neurohumor and for its relation to LSD-induced psychosis and to clinical psychoses. These authors have suggested that mental disturbances following the administration of small amounts of LSD may arise because of (1) the antimetabolic action of LSD on 5-hydroxytryptamine (5HT) or serotonin, thus diminishing the content of this substance in the brain, or (2) by increasing the amount of 5HT in the brain by competing for amine oxidase, thus permitting the accumulation of abnormally large amounts of 5HT in the brain. In terms of these hypotheses these investigators further postulate that endogenous psychoses such as schizophrenia may result from either too much or too little 5HT in the brain. Woolley and Shaw present no direct evidence for either of these hypotheses, but they marshal an impressive array of indirect evidence in favor of 5HT as a potent neurohumor that acts as an antimetabolite to LSD in relation to its action on smooth muscle.

Gaddum[40] has independently made suggestions similar to those of Woolley and Shaw. He and his collaborators have demonstrated that the brain, especially the hypothalamus, contains considerable amounts of 5HT.[41] Twarog and Page[42] have also demonstrated the presence of this substance in brain. Recently Brodie, Pletscher, and Shore[43] have shown that reserpine reduces the amount of 5HT in the brains of animals and also reduces the larger store of 5HT in blood platelets. Accompanying the administration of reserpine, quantities of 5-hydroxyindoleacetic acid, the principal metabolite of 5HT, appear in the urine.[44] The 5HT is released from cellular storage, and this effect has also been demonstrated by its release from platelets *in vitro*.[45] Reserpine rapidly enters the brain, reaches a maximum in rabbits in 10 to 15 minutes, and disappears entirely in 8 hours. The sedative effects of reserpine, however, last about 48 hours. The 5HT was found to be released almost completely from brain depots in 4 hours, and it remained at a constant low level for 30 hours, and then it rose to normal values in 7 days.[46] The investigators Hess, Shore, and Brodie[46] conclude that reserpine produces its central effects by causing changes in 5HT.

A product of *in vitro* degradation of 5HT is bufotenin [5-hydroxy-3-(2 dimethylammoethyl)-indole]. Bufotenin, the *N*-dimethyl derivative of 5HT, has been reported by Evarts[47] to produce bizarre behavior in monkeys following its injection. Both LSD and bufotenin in doses of 1.0 to 5.0 mg./kg. administered intravenously produce a marked degree of tameness in monkeys, together with gross sensory disorders, but without causing any defect in muscle power. H. D. Fabing, in a personal communication, reports that it acts in relatively large doses to produce psychoticlike behavior in man. Bufotenin has also been identified[48] as the active principle of the cohoba snuff, obtained from seeds of the plant *Piptadenia peregrina*, that has been used by necromancers and priests of some South American Indian cults for many centuries in their religious rites.

While 5HT that has been injected penetrates the blood-brain barrier to only a small degree, injected 5-hydroxytryptophan, a precursor of 5HT, does pass through the blood-brain barrier readily and has been found by Udenfriend, Bogdanski, and Weissbach[49] to elevate the content of 5HT of the brains of animals as much as twentyfold. When this happens, cats behave in the bizarre fashion seen following administration of LSD or bufotenin. The enzyme 5-hydroxytryptophan decarboxylase that catalyzes the formation of serotonin is not affected by reserpine. Serotonin continues to accumulate in the presence of reserpine, but it does so in the free form.[50] Thus there is evidence that abnormal accumulation of bound 5HT in the brain may produce psychoses and that the tranquilizing action of reserpine, so useful in current psychiatric practice, may be mediated through its effect on reducing the amount of 5HT in the brain by freeing it from a bound form so that it is metabolized by amine oxidase. These matters are discussed by Kuntzman *et al.*[50]

Unpublished work of Alan Slocombe in our laboratory indicates that LSD inhibits the destruction of serotonin by amine oxidase in homogenates of rat brain. The inhibition compares favorably with that of marsilid (isonicotinylisopropyl hydrazine), an inhibitor of amine oxidase. Both are about equally active in concentrations of the order of 10^{-3}M. These data support the foregoing view that LSD may cause accumulation of serotonin in restricted sites in the brain by partially blocking the destruction of serotonin in competing with it for monamine oxidase.

Summary

This paper reviews the biochemical effects of LSD and some similar drugs, particularly with reference to several quite different hypotheses that have been proposed concerning the biochemical determinants of clinical psychoses, especially schizophrenia.

(1) Some abnormalities of phosphorous metabolism in clinical schizophrenia and in normal persons under the influence of LSD are considered in terms of a hypothesis that schizophrenia may be due to the production of an endogenous metabolite that both produces psychosis and modifies phosphorylation after the manner of LSD. The question is an open one as to whether the derangements in phosphate metabolism are causal to the psychosis or are coincidental only.

(2) The hypothesis that clinical psychosis may be due to disturbance in metabolism of catechol amines, perhaps in the form of a metabolite of epinephrine or norepinephrine, is reviewed. Also considered in this connection are possible abnormalities of metabolite formation from serotonin. Adrenochrome and adrenolutin, both derivatives of epinephrine, and bufotenin, a derivative of serotonin, have been reported to produce psychotic episodes.

(3) The hypothesis that clinical psychoses may arise from too much or too little serotonin in the brain and that LSD acts to modify the serotonin content is discussed. The view that reserpine exerts its tranquilizing action by reducing brain serotonin is also considered.

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A REVIEW OF THE BIOCHEMICAL EFFECTS *IN VITRO* OF CERTAIN PSYCHOTOMIMETIC AGENTS

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The amount of literature on the biochemical effects *in vitro* of the psychotomimetic agents is quite limited. Perhaps this is because of the relatively recent rise to popularity of this class of drugs or because most investigators of the subject have not yet found effects that they feel to be of sufficient significance to warrant publication. Some purpose may be served, however, by collecting this limited data into one place, if only to emphasize its meagerness.

The earliest reference that was found dealing with *in vitro* biochemical effects of a psychotomimetic agent concerns the drug mescaline. In 1933, Quastel and Wheatley, as a part of a rather extensive study on the effects of amines on brain oxidations, reported that mescaline inhibited the oxidation of glucose, lactate, pyruvate, and glutamate (but not of succinate) by minces of guinea pig brain. Under the somewhat special conditions of allowing the mince to incubate in the presence of inhibitor and in the absence of substrate for from 2 to 3 hours, the oxidation of the above substrates added at the end of the incubation period was inhibited approximately 65 per cent by 4×10^{-3} M mescaline. At that time Quastel and Wheatley suggested that perhaps malfunction of the liver might lead to faulty detoxification of amines that might have untoward effects on brain respiration, a condition that in turn might be the basis for certain mental abnormalities. Quastel and Wheatley reported similar inhibitions in experiments with quite a variety of amines and, perhaps for this reason, their observations on mescaline have not been given more prominence.

Subsequent investigations of mescaline are summarized in TABLE 1. The first line of the table deserves some comment. This very approximate figure is derived, as are similar figures in subsequent tables, by assuming the uniform distribution of the usually effective dose (in this instance 500 mg.) throughout a 70-kg. man and rounding off the resulting calculated concentration to the nearest even power of 10. The value cannot be defended in the absence of actually determined tissue concentrations except to say that it is no doubt a maximum value for the concentration of mescaline in the central nervous system resulting from that dose. In this regard the C^{14}-labeled mescaline distribution data of Block, Block, and Patzig (1952a) show that only a small fraction of the administered label appears in the central nervous system. On that score, then, the concentrations studied by Quastel and Wheatley are much above those expected to occur *in vivo* from the administration of mescaline. The faulty functioning of the liver, of course, might lead to the accumulation of considerable concentrations of toxic amines.

The findings of Quastel and Wheatley were re-examined in 1948 by Schueler, whose results were essentially the same as Quastel and Wheatley's, but Schueler also emphasizes the necessity for preincubation and the disparity between effective *in vivo* and *in vitro* concentrations. Woodford (1952) has apparently

TABLE 1

SUMMARY OF THE *in Vitro* BIOCHEMICAL EFFECTS OF MESCALINE

System	Effect	Concentration	Reference
Psychotomimetic effects *in vivo*		10^{-5} M	
Glucose, lactate, pyruvate, glutamate (but not succinate) oxidation by guinea pig brain minces	65% inhibition	4×10^{-3} M	Quastel & Wheatley, 1933
Glucose, lactate, pyruvate (but not succinate) oxidation by rat brain minces	55% inhibition	4×10^{-3} M	Schueler, 1948
Oxaloacetic & oxalosuccinic carboxylase, transaminase	No effect		Block, Block & Patzig, 1952b
Succinic dehydrogenase and cytochrome oxidase	No effect	10 mM	Clark *et al.*, 1954
Pyruvate oxidation by brain homogenates	42% inhibition	10 mM	Clark *et al.*, 1954
Glucose oxidation in electrically stimulated guinea pig brain slices	50% inhibition	1×10^{-3} M	Lewis & McIlwain, 1954
Lactate production in electrically stimulated guinea pig brain slices	50% inhibition	1×10^{-3} M	
Glucose oxidation in unstimulated guinea pig brain slices	No effect	1×10^{-2} M	
Lactate production in unstimulated guinea pig brain slices	No effect	1×10^{-2} M	
Oxidative phosphorylation in rat brain mitochondria	No effect	1×10^{-3} M	Bain, 1955

also confirmed the findings of Quastel and Wheatley, and he has again stressed the preincubation conditions. While it may not be strictly germane to the matter under discussion, I believe that it is appropriate to point out that Schueler, acting upon the information that the oxidation of succinate was not inhibited *in vitro*, tried to reverse mescaline hallucinations by the intravenous administration of sodium succinate. Apparently, these experiments were successful, and the hallucinations induced by mescaline were indeed cut short by the succinate. Unfortunately, Schueler did not control his experiments with adequate placebos either in the form of saline or, more appropriately perhaps, in the form of 1 of those substrates whose oxidation was inhibited *in vitro* by mescaline. I suggest this point as one that some adequately prepared group might wish to re-examine. Osmond (1955) makes reference to unpublished observations on it.

We come next to 2 groups of investigators with negative reports: Block, Block, and Patzig (1952b), reporting that mescaline was without effect on oxaloacetic or oxalosuccinic carboxylases or transaminase, and Clark *et al.* (1954) failing to find any effect on succinic dehydrogenase or cytochrome oxidase. The latter authors, however, did report that mescaline inhibits the oxidation of pyruvate by homogenates of brain tissue.

McIlwain has devised a very elegant method for stimulating excised tissue preparations electrically in the Warburg vessel. He finds that such stimulation results in an increase in the uptake of oxygen by brain tissue respiring in various substrates. Utilizing this test system, Lewis and McIlwain (1954) were unable to demonstrate any effect of 10^{-2} M mescaline on the uptake of oxygen or the production of lactate on a substrate of glucose by unstimulated slices of guinea pig brain. Stimulation, however, revealed that 10^{-3} M mescaline could produce 50 per cent inhibition of the extra uptake of oxygen and the formation of lactate resulting from the electrical pulses. In general, the stimulated metabolism is more sensitive to inhibitors than is the unstimulated metabolism. When the concentration of mescaline was reduced to 10^{-4} M, its inhibitory effect on even the stimulated metabolism was nil. This concentration is still 10 times that calculated to occur *in vivo*.

Preparations of mitochondria from various tissues have become favorite test objects for *in vitro* investigations, particularly for oxidative phosphorylation. Mescaline at 10^{-3} M had no effect on either the uptake of oxygen or on the associated phosphorylation in preparations of mitochondria of the brains of rats respiring *in vitro* on a pyruvate substrate (Bain, 1955).

It may be seen that the investigations on mescaline to date have been confined to carbohydrate-transforming enzymes. Even cholinesterase has not been tested directly so far as I can ascertain. It is of interest, however, that the brains of animals treated with mescaline show no change in the content of acetylcholine (Poloni and Maffezzoni, 1952).

Lysergic acid diethylamide (LSD) has also been studied primarily with respect to the metabolism of carbohydrates. The information is summarized in TABLE 2. Again the calculated *in vivo* concentration is given and again, in the light of C^{14}-labeled LSD distribution studies (Stoll *et al.*, 1955), this must be a maximum concentration.

In 1953 Mayer-Gross, McAdam, and Walker observed that in subjects treated with LSD there was a small fall in blood sugar and a concomitant rise in blood hexose monophosphate. Following up this lead these investigators performed experiments *in vitro* using homogenates of guinea pig brain and reported that 4×10^{-9} M LSD produced a 30 per cent stimulation of the oxidation of glucose and a 40 per cent inhibition of the utilization of hexose monophosphate. With homogenates of liver a 10 per cent inhibition was observed in both cases. It is not clear just which hexose monophosphate was accumulating in these experiments, since the method that was used for its measurement was designed for the determination of the old "Embden ester," which was a mixture of monophosphates. Assuming that it is glucose-1-phosphate or glucose-6-phosphate, the above results are difficult to interpret because the accumulation of either of these 2 phosphates implies a block of both the Embden-Meyerhof shunt and the pentose shunt which are the main pathways for the breakdown of glucose, and yet there was stated to be an increase in the oxidation of glucose. Since glucose itself was not directly determined in the *in vitro* experiments, it is conceivable that it was the oxidation of some other substance that was accelerated and that the breakdown of glucose was actually blocked. It is noteworthy that these effects were observed at exceedingly low concentrations of LSD that approach rather closely those concentrations presumed to be present *in vivo*.

Unfortunately, the results obtained by Mayer-Gross, McAdam, and Walker have not been confirmed by later workers. Lewis and McIlwain (1954) observed no effect of LSD on the oxidation of glucose by slices of guinea pig

TABLE 2

SUMMARY OF THE *in Vitro* BIOCHEMICAL EFFECTS OF LSD

System	Effect	Concentration	Reference
Psychotomimetic effects *in vivo*		10^{-9} M	
Glucose oxidation by guinea pig brain homogenate	30% stimulation	4×10^{-9} M	Mayer-Gross, McAdam & Walker, 1953
Glucose oxidation by guinea pig liver homogenate	10% inhibition	4×10^{-9} M	
Hexose monophosphate utilization by guinea pig brain homogenate	40% inhibition	4×10^{-9} M	
Hexose monophosphate utilization by guinea pig liver homogenate	10% inhibition	4×10^{-9} M	
Glucose oxidation in electrically stimulated guinea pig brain slices	40% inhibition	5×10^{-5} M	Lewis & McIlwain, 1954
Lactate production in electrically stimulated guinea pig brain slices	40% inhibition	5×10^{-5} M	
Glucose oxidation in unstimulated guinea pig brain slices	No effect	1×10^{-4} M	
Lactate production in unstimulated guinea pig brain slices	10% inhibition	1×10^{-4} M	
Succinic dehydrogenase from brain	23% inhibition	1 mM	Clark *et al.*, 1954
Cytochrome oxidase from brain	13% stimulation	1 mM	
Glucose oxidation by rat brain homogenate	No effect	1×10^{-6} M	Bain & Hurwitz, 1954
Glucose oxidation by rat liver homogenate	No effect	1×10^{-6} M	
Glucose oxidation by unstimulated rabbit brain slices	10% inhibition	3×10^{-5} M	Bain & Morrison, 1955
Glucose oxidation by dinitrophenol stimulated rabbit brain slices	10% inhibition	3×10^{-5} M	
Oxidative phosphorylation in rat brain or liver mitochondria	No effect	1×10^{-4} M	Bain, 1955
Pseudocholinesterase human plasma and brain	50% inhibition	5×10^{-6} M	Thompson, Tickner & Webster, 1955
True cholinesterase human erythrocytes and brain	10% inhibition	5×10^{-5} M	

brain and only minimal inhibition of the accumulation of lactic acid at 10^{-4} M. The extra uptake of oxygen and the production of lactic acid on glucose resulting from electrical stimulation of the slices were inhibited 40 per cent by LSD at 5×10^{-5} M. Clark *et al.* (1954) reported inhibition of succinic dehydrogenase and stimulation of cytochrome oxidase from brain tissue. We were unable to repeat the experiments of Mayer-Gross, McAdam, and Walker using homogenates of the tissues of rats and concentrations of LSD up to 10^{-6} M (Bain and Hurwitz, 1954). Only slight inhibitions of the oxidation of glucose by slices of rabbit brain were observed at 3×10^{-5} M LSD even with slices whose respiration had been stimulated by dinitrophenol (Bain and Morrison, 1955). Mitochondrial preparations of brain tissue are not sensitive to LSD either with respect to the uptake of oxygen or associated phosphorylations with concentrations of the drug up to 10^{-4} M (Bain, 1955).

It is of some interest that Lewis and McIlwain (1954) attempted to reverse with serotonin the inhibition by LSD of electrically stimulated slices of brain and were unsuccessful in this attempt. It may be mentioned in passing that serotonin is a weak uncoupling agent for the process of oxidative phosphorylation producing 50 per cent uncoupling with mitochondria of liver at 5×10^{-4} M (Bain and Hurwitz, 1953). Again, because of the wide difference in concentrations effective *in vivo* and those effective *in vitro*, this last-mentioned effect probably has little physiological significance.

Following up a report by Poloni and Maffezzoni (1952) that LSD causes an increase in the level of acetylcholine in the brain of the guinea pig, Thompson, Tickner, and Webster investigated the effect of LSD on cholinesterase. They found a 50 per cent inhibition of the pseudoesterase from the brain of the human at 5×10^{-6} M, but 10 times this concentration produced only a 10 per cent inhibition of the true esterase that is presumably the enzyme involved most directly with nerve function. The same sensitivities were found to hold for the true esterase and the pseudoesterase of the blood. A number of other alkaloids of ergot were also tested against the serum pseudoesterase, and only ergometrine approaches LSD in potency as an inhibitor of the serum pseudoesterase. Even this alkaloid was only one fifth as potent as LSD. Human esterases were found to be appreciably more sensitive to the inhibitory properties of LSD, and the authors also comment upon the relatively strong specificity of LSD for 1 (the pseudoesterase) of the 2 types of esterases in contrast to other well-known inhibitors that, while showing a considerable degree of specificity, do not reveal one nearly as marked as that observed with LSD. Again serotonin had no effect on the inhibitory properties of LSD, although inhibition of the esterase by LSD could be shown to be reversible by appropriate dilution experiments. While the inhibition of pseudoesterase may possibly explain the accumulation of acetylcholine observed by Poloni and Maffezzoni in the brains of animals treated with LSD, the authors show considerable reluctance to attribute any great significance to their findings with respect to mental disease. Again, the effects are observed at concentrations 1000 times those calculated to occur *in vivo*.

Adrenochrome, or perhaps its derivative, adrenoxine, has been implicated recently as a psychotomimetic. In 1937, Green and Richter, in studying the effect of epinephrine on certain oxidative systems, found that its oxidation product, adrenochrome, could interact with lactic and malic dehydrogenases as a hydrogen carrier at concentrations as low as 6×10^{-7} M. It was in this same year that Heirman (1937) reported the physiological effects of adrenoxine. In 1946, Randall reported that adrenochrome was a potent inhibitor of glycolysis in preparations of brain tissue, and Meyerhof and Randall (1948) showed 2 years later that this inhibition could be attributed to the inhibiton of hexokinase and phosphohexokinase (TABLE 3). Fifty per cent inhibition of their system from extracts of rabbit brain was obtained with 4×10^{-5} M adrenochrome. The degree of inhibition was an inverse function of the amount of adenosine triphosphate present.

Issekutz *et al.* (1950) reported that epinephrine and norepinephrine produce marked increases in the metabolism of isolated striated muscle, but could find no effect of adrenochrome up to 10^{-5} M. Woodford (1952), however, is reported by Hoffer, Osmond, and Smythies (1954) and Osmond (1955) to have observed

TABLE 3

SUMMARY OF THE *in Vitro* BIOCHEMICAL EFFECTS OF ADRENOCHROME

System	Effect	Concentration	Reference
Psychotomimetic effects *in vivo*		10^{-6} M	
Hydrogen transport	Stimulation	6×10^{-7} M	Green & Richter, 1937
Glycolysis in rat brain homogenate	Inhibition	4×10^{-6} M	Randall, 1946
Hexokinase and phosphohexokinase in rabbit brain extracts	50% inhibition	4×10^{-5} M	Meyerhof & Randall, 1948
Metabolism of isolated striated muscle	No effect	1×10^{-5} M	Issekutz *et al.*, 1950
Glycolysis and intermediary carbohydrate metabolism	Inhibition		Woodford, 1952
Ascorbic acid oxidation by liver homogenate	Stimulation	1×10^{-6} M	Radmsa & Golterman, 1954
Lactate : DPN : ascorbic : homogenate	Inhibition	1×10^{-4} M	

powerful inhibitory effects of adrenochrome (or some breakdown product thereof) on glycolysis and intermediary carbohydrate metabolism of preparations of brain tissue *in vitro*, although the details of these studies have never been formally published so far as I am aware. Radmsa and Golterman (1954) have studied again the activity of adrenochrome in hydrogen-transport systems involving ascorbic acid, coenzyme 1, lactate, and enzymes from homogenates of liver. These authors observed the stimulation of the oxidation of ascorbic acid by 10^{-6} M adrenochrome and inhibition of the oxidation of lactates by 10^{-4} M. In a recent study Park, Meriwether, and Park (1956) found that 5×10^{-4} M adrenochrome completely uncouples oxidative phosphorylation in hamster-liver mitochondria respiring on β-hydroxybutyrate. This effect is not reversed by magnesium, in contrast to the uncoupling effect of thyroxin (Bain, 1954). Subeffective amounts of thyroxin (5×10^{-5} M) and adrenochrome (2×10^{-5} M) when added together, however, produced about 50 per cent uncoupling.

There appears to be some doubt as to the actual identity of the psychotomimetic agent in preparations of adrenochrome. It is perhaps noteworthy, however, that some of the *in vitro* effects of adrenochrome are observed with concentrations that approximate, at least, those that conceivably might occur *in vivo* with the doses reported to be hallucinogenic.

A few isolated observations on other psychotomimetic substances may be mentioned. Boriani (1951) has reported that bulbocapnine inhibits cholinesterase (the original publications were not available to this reviewer). Bufotenin also has been reported to be a very active inhibitor of cholinesterase (Augustinsson, 1950), as has ibogaine (Augustinsson, 1948). In contrast to its demethylated analogue, serotonin, bufotenin is not an inhibitor of oxidative phosphorylation in preparations of brain mitochondria at concentrations as great as 10^{-4} M, although perhaps higher concentrations might have been effective (Bain, 1955).

It may be seen from this brief review that we have really very little evidence of an *in vitro* biochemical nature on which to base any hypothesis as to the mechanism of action of these agents. While there is a considerable amount of speculation that the effects of the psychotomimetic drugs are to be explained by a hypothetical action upon some metabolic process, the data so far available have done little to identify this process. With the best established and most potent agent of this class, LSD, the only *in vitro* result that comes reasonably close to meeting the "concentration *in vitro* versus concentration *in vivo*" criteria are the observations of Mayer-Gross, McAdam, and Walker on the metabolism of carbohydrates. These reports, unfortunately, have not been confirmed.

There are, of course, many as-yet-unexplored possibilities that need to be tested. The metabolic pathways leading to the production or destruction of serotonin, epinephrine, or even acetylcholine have not received adequate attention. Mechanisms of ion transport and other processes concerned with the function of nerve cells are not yet mentioned with reference to the action of drugs of this class. In view of the high dilution at which these agents are effective and the peculiarly selective type of functional derangement that they produce it would seem that the technically difficult problem of the investigation of effects in restricted areas of the central nervous system will be even more urgent with this class of agents than with the more generally acting narcotics and convulsants. In addition, we cannot ignore the possibility that some as-yet-undiscovered reaction or system of reactions will prove to be the key to the biochemical mode of action of drugs of this class.

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Discussion of the Paper

Roscoe O. Brady (*Section on Lipid Chemistry, Laboratory of Neurochemistry, National Institute of Neurological Diseases and Blindness*): I was interested to learn of James A. Bain's findings with regard to the apparent lack of an effect of LSD on the metabolism of glucose by brain. In my laboratory at the National Institute of Neurological Diseases and Blindness we have observed that the addition of LSD to brain homogenates has little if any effect upon the conversion of C^{14}-labeled glucose to carbon dioxide. Similar results were obtained in experiments in which the conversion of labeled acetate to $C^{14}O_2$ was examined. These findings seem to indicate that LSD has no direct effect on glycolysis, acetate activation, or oxidation via the tricarboxylic acid cycle in these gross preparations. Furthermore, added LSD had no effect upon the synthesis or amide transfer reaction of glutamine catalyzed by a partially purified brain enzyme preparation.

An experiment was designed to test the possible N-acetylation of LSD using acetyl coenzyme A as acyl donor similar to the formation of N-acetylimidazole described by Stadtman and White.[1] It was observed that LSD could indeed serve as an acetyl acceptor in an enzyme system obtained from extracts of *Cl. kluyveri*. Although the product of the reaction has not been isolated, if N-acetyl-LSD is formed, it is likely that spontaneous decomposition or transacetylation to compounds such as cysteine could attenuate the supply of biologically important acetyl coenzyme A. Before such a hypothesis can be considered seriously, however, the demonstration of this reaction in preparations of brain tissue is obligatory, and so far the attempts made in this regard have been unsuccessful.

I further agree with Bain that it is most desirable to examine the effect of LSD on the enzymatic activity of selected neural cells. I am afraid it is quite naive to expect to find a gross enzymatic alteration in brain extracts if the abnormalities caused by LSD are restricted to a few specific cells within areas such as the lateral geniculate body or the retina. I believe it is now imperative to examine the enzymatic patterns of selected types of cells from these regions by techniques similar to the exquisite methods perfected by Oliver H. Lowry and his collaborators. Unless these practices are adopted, the observance of a specific enzymatic lesion caused by LSD is likely to be a highly fortuitous event.

Reference

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Harry Goldenberg (*Department of Biochemistry, Hillside Hospital, Glen Oaks, N. Y.*): I should like to supplement the material presented by James A. Bain with the results of experiments that Vivian Goldenberg and I carried out last year at the Hillside Hospital. A number of hallucinogens and other amines were tested for their inhibitory action on human serum cholinesterase, using acetylcholine as substrate. It was necessary to employ mescaline in concentrations of 10^{-2} M in order to produce significant inhibition, which answers the question you raised before. On the other hand, LSD and brom-LSD proved to be potent inhibitors. The relative potency of the various compounds followed the order: eserine > LSD > brom-LSD > neostigmine > LAE (d-lysergic acid monoethylamide) > chlorpromazine > serotonin $\cong$ tryptamine > mescaline. The I_{50} values for the first 5 alkaloids, that is, the concentrations required to produce a 50 per cent inhibition under our experimental conditions, were as follows: eserine, 1×10^{-6} M; LSD, 3×10^{-6} M; brom-LSD, 8×10^{-6} M; neostigmine, 1×10^{-5} M; LAE, 2×10^{-4} M. Adrenochrome prepared in our laboratory was somewhat more inhibitory than mescaline, but less so than serotonin. These data suggest that there is no clear-cut correlation between the psychic disturbances produced by drugs and their ability to inhibit pseudocholinesterase, for while brom-LSD readily blocks the enzyme, it does not produce schizogenic effects at all comparable to those of LSD.[1] Further, chlorpromazine is more inhibitory than mescaline yet it is a tranquilizing agent that is reported to counter psychotomimetic action. In this connection I might add that neither chlorpromazine nor Frenquel reverses the inhibition of serum cholinesterase produced by LSD.

Despite the uncertain role of pseudocholinesterase in the psychotomimetic process, its ready inhibition by LSD offers useful application in analysis. We have been able to detect as little as 0.2 μg. of LSD added to 100 ml. of urine by first extracting the alkaloid with ethylene dichloride, transferring the LSD into acid and thence back into solvent, evaporating it to dryness, and then incubating it with serum and substrate. Recoveries of from 40 to 50 per cent have been obtained.

Reference

1. Cerletti, A. & E. Rothlin. 1955. Role of 5-hydroxytryptamine in mental diseases and its antagonism to lysergic acid derivatives. Nature. **176:** 785–786.

Ernst Rothlin (*University of Basel, Basel, Switzerland*): James A. Bain has alluded to the investigations of Thompson, Tickner, and Webster, who found that lysergic acid diethylamide (LSD) had no effect upon true cholinesterase, while they did find some action on pseudocholinesterase. We have confirmed these findings. A new finding in our experiments is that brom-LSD behaves in the same way that LSD does in that it exercises an inhibitory action on pseudocholinesterase, but not on true cholinesterase. The effective concentrations for LSD and brom-LSD are the same. No significant effect on aminoxidase was found for LSD or brom-LSD when using tyramine as the substrate.

Reference

Thompson, R. H. S., A Tickner & G. R. Webster. 1955. Brit. J. Pharmacol. **10:** 11.

THE EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE ON CEREBRAL CIRCULATION AND OVER-ALL METABOLISM

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d-Lysergic acid diethylamide (LSD-25), a partially synthesized derivative of ergot, has recently been found to produce in minute doses marked aberrations in psychological and mental functions.[1, 2, 3] Because these disturbances simulate to some degree those observed in naturally occurring psychoses, there has been considerable interest in its mode of action. It has been suggested that the psychotomimetic action of lysergic acid is related to its reported antagonism to 5-hydroxytryptamine (serotonin),[4, 5, 6] a compound normally present in the brain.[7] Since serotonin is known to have powerful vasoconstrictor properties, it is conceivable that the antagonistic action of lysergic acid might be reflected in changes in the cerebral circulation. Furthermore, since the normal functions of the brain are completely dependent on a continuously obligatory consumption of oxygen and glucose, it might be supposed that disorders in the biochemical processes underlying the functions so grossly disturbed by LSD-25 would be reflected in alterations in the oxygen and glucose utilization by the brain. Mayer-Gross, McAdam, and Walker[8, 9] have found both *in vitro* and *in vivo* evidence for disturbances in carbohydrate metabolism produced by LSD-25. An increase in circulating hexosemonophosphate and glucose *in vivo* and a decreased breakdown of hexosephosphate in brain and liver *in vitro* have led these investigators to postulate that LSD-25 causes an increased metabolism of glycogen coupled with a block in the breakdown of hexosemonophosphate. Studies of the effects of lysergic acid on cerebral oxygen consumption have previously been done only *in vitro*, and these have led to contradictory results.[9-12]

In order to determine the effects of LSD-25 on the cerebral circulation and oxygen and glucose metabolism of individuals actively demonstrating the behavioral changes produced by the drug, measurements of these and related functions were undertaken in normal or nonpsychotic, conscious human subjects. Also, in consideration of the remote possibility that schizophrenic patients might react differently to the drug, similar studies were performed in a group of such patients.

Method

Cerebral blood flow (CBF) was measured by means of the nitrous oxide method[13] in 13 normal or nonpsychotic subjects and 9 chronic schizophrenic patients during a resting or control period and again during the height of action of intravenously administered LSD-25.* Eleven of the normal subjects were conscientious objectors or members of a religious sect who had volunteered to be experimental subjects in medical research. Of the remaining 2, both of whom had volunteered for this specific procedure, one (Rb. M.) was a normal working employee and the other (J. K.) a patient whose diagnosis was "anxiety neurosis." The schizophrenic patients were of most major types and had been selected from a hospitalized population on the basis of their anticipated cooperativeness during the procedure. Some of the patients, as well as the normal subjects, had been given trial tests with LSD-25 no less than 1 week previously in order to determine their responsiveness and the nature of their response to the drug. This screening was done to favor the occurrence of a definite reaction, but one not too violent to interfere with the procedure during the cerebral blood flow studies. The duration of the disease in the schizophrenic patients ranged from 5 to 22 years, and all exhibited mild to moderate deterioration. In addition to psychotherapy, almost all of the patients had received some form of shock or drug therapy, but in no case during the year preceding the time of these studies.

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* LSD-25 supplied in 1 cc. ampules containing 0.1 mg. per cc. by Sandoz Chemical Works, Inc., Hanover, N. J.

Each study consisted of two determinations of cerebral blood flow and associated functions, a control measurement followed by an experimental determination during the drug action. Immediately following the control measurements, the symptom questionnaire designed by Abramson and his co-workers[14] was administered to all the normal subjects and to those schizophrenic patients who were communicative. Following the completion of the questionnaire, 120 μg. of LSD-25 were injected intravenously in all but one normal subject (M. G. L.) and one schizophrenic patient (V. S.), in whom the dose injected was 100 μg. Administration of the symptom questionnaire was repeated at approximately 15, 40, 105, and 165 minutes following the drug injection. Because the mean number of symptoms appeared to be maximum at the 40-minute point, the experimental measurements of cerebral blood flow and associated functions were made at that time.

Mean arterial blood pressure (MABP) was measured with an air-damped mercury manometer connected by plastic tubing to the femoral arterial needle. Mean internal jugular venous pressure (MJVP) was determined by means of a Statham P23B strain gauge connected to the internal jugular venous needle and used in conjunction with a Brush Universal amplifier and oscillograph, the former modified to permit electrical integration of the venous pulse for the determination of mean pressure. Blood oxygen and carbon dioxide contents were determined by the manometric method of Van Slyke and Neill.[15] Arterial hemoglobin concentration was measured photometrically by a modification of the method of Evelyn and Malloy.[16] Blood glucose concentrations were determined as the mean of triplicate determinations by the Nelson method.[17] Blood pH was measured anaerobically at ambient temperature by means of the MacInnes-Belcher glass electrode and Cambridge Model R Potentiometer and was corrected to 37° C. by means of the factors of Rosenthal.[18] Blood oxygen saturation was determined spectrophotometrically by means of a modification of the method of Wyeth, Ecker, and Polis.[19] Blood carbon dioxide tension (pCO_2) was computed by means of the nomograms of Peters and Van Slyke.[20] Calculations of cerebral blood flow (CBF), oxygen consumption (CMR_{O_2}), glucose utilization (CMR_G), vascular resistance (CVR), and respiratory quotient (R.Q.) were made as previously described.[13]

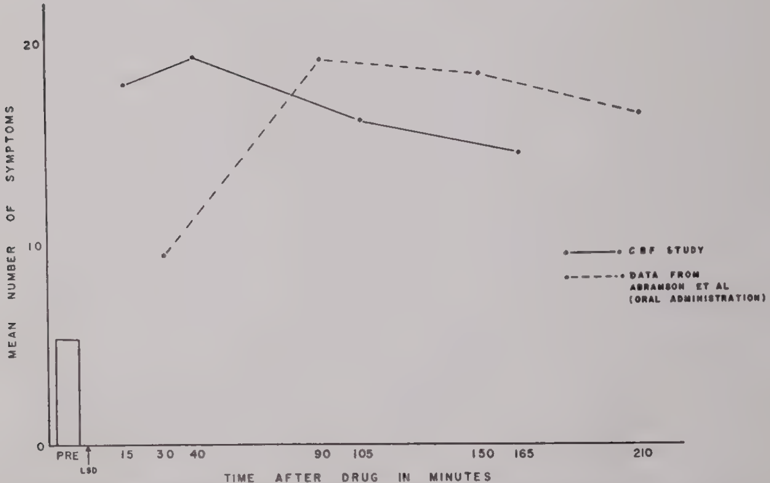

FIGURE 1. Graphic illustration of responses to symptom questionnaire of Abramson *et al.*[14] following oral and intravenous administration of LSD-25.

Results

In FIGURE 1 are graphically represented the results obtained in normal subjects with the symptom questionnaire when administered before and at various times subsequent to the intravenous injection of LSD-25. The results obtained by Abramson and his co-workers[14] following oral administration of the drug are similarly plotted for comparison. It is clear from FIGURE 1 that in normal subjects intravenous administration of LSD-25 does not result in any greater symptom formation than does oral administration, but the peak mean number of symptoms occurring at approximately 40 minutes following injection of the drug precedes the peak achieved with oral administration by nearly an hour. For this reason, the intravenous route of administration was chosen in these studies, and experimental data on cerebral circulation and metabolism and related functions were collected at approximately the 40-minute point. Since the symptom questionnaire could not be satisfactorily administered to schizophrenic patients, it was assumed that the timing of the LSD-25 effect was similar in this group, and the same schedule of experimental measurements was followed as in the normal subjects.

In TABLES 1, 2, and 3 are presented the data on the cerebral circulation and metabolism and related functions obtained in both the normal subjects and the schizophrenic patients. In both groups LSD-25 failed to produce any statistically significant change in pulse rate, respiratory rate, or oral temperature, although the change in pulse rate in the normal subjects from a mean control value of 78 to a mean experimental value of 91 beats per minute was only barely short of statistical significance ($p < 0.1 > 0.05$). In both normal subjects and schizophrenic patients slight but statistically significant in-

TABLE 1

EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON PULSE, RESPIRATION, AND BODY TEMPERATURE

Subject	Sex	Age	Interval in min.	Dose in µg.	Pulse per min.		Respiratory rate per min.		Oral temp. °C.	
					I	II	I	II	I	II
Thirteen Normal Subjects										
M. G. L.	M	19	91	100	68	78	18	20	36.2	—
W. W.	M	28	127	120	74	110	15	18	36.8	37.3
Rb. M.	M	30	100	120	82	80	21	24	36.6	36.6
A. R.	M	19	110	120	58	56	18	21	36.9	36.8
J. K.	F	46	120	120	83	80	19	16	36.6	36.2
D. B.	M	24	107	120	80	82	16	17	36.9	36.7
M. L.	F	22	150	120	90	85	20	17	36.8	36.7
L. N.	F	24	138	120	99	114	18	21	37.4	37.2
H. H.	M	25	116	120	76	166	10	14	37.4	37.5
C. S.	F	18	111	120	71	80	18	20	37.1	36.8
Rn. M.	M	19	127	120	77	84	14	16	36.7	37.2
D. H.	M	19	100	120	72	78	22	20	36.6	36.9
M. G.	F	18	129	120	88	94	16	10	37.5	36.7
Mean	—	24	117	—	78	91	17	18	36.9	36.9
Standard error	—	±2	±5	—	±3	±7	±1	±1	±0.1	±0.1
*p**	—	—	—	—	<0.1	>0.05	>0.4		>0.6	
Nine Schizophrenic Patients										
V. S.	F	41	108	100	76	96	20	22	36.9	—
T. H.	M	36	95	120	92	92	22	20	36.3	36.9
R. T.	M	22	82	120	60	84	16	12	36.6	—
P. L.	M	33	82	120	104	105	14	15	37.0	37.0
J. W.	M	33	128	120	92	82	8	14	37.0	37.0
W. H.	M	27	113	120	76	78	19	26	36.8	37.3
W. C.	M	34	76	120	101	87	27	23	37.0	36.9
B. G.	M	31	112	120	102	119	32	24	36.6	38.2
D. L.	M	27	80	120	94	102	20	21	37.0	37.3
Mean	—	32	97	—	89	94	20	20	36.8	37.2
Standard error	—	±2	±6	—	±5	±5	±2	±2	±0.1	±0.2
*p**	—	—	—	—	>0.2		>0.9		>0.1	

I: control.
II: during period of LSD-25 effect.
* Determined by the method of paired comparison.

creases in mean arterial blood pressure (MABP) were produced by the drug rising from 86 to 91 mm. Hg ($p < 0.05$) in the former and from 90 to 97 mm. Hg ($p \sim 0.02$) in the latter. Mean internal jugular venous pressure (MJVP) was unaffected in the normal subjects but in the schizophrenic patients was raised significantly from 7 to 9 mm. Hg ($p \sim 0.02$) by the drug. The effects of LSD-25 on the various cerebral circulatory and metabolic functions studied, for example, cerebral blood flow (CBF), vascular resistance (CVR), oxygen consumption (CMR_{O_2}), glucose utilization (CMR_G), arteriovenous oxygen and glucose differences [$(A\text{-}V)_{O_2}$ and $(A\text{-}V)_G$, respectively], and R.Q. were remarkable only for the absence of any statistically significant changes.

TABLE 2

EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON CEREBRAL CIRCULATION AND METABOLISM

Subject	MABP in mm. Hg		MJVP in mm. Hg		CBF in cc./100gm./min.		CVR in $\frac{\text{mm. Hg}}{\text{cc./100gm./min.}}$		(A-V)$_{O_2}$ in vol. %		CMR$_{O_2}$ in cc./100gm./min.		(A-V)$_G$ in mg.%		CMR$_G$ in mg./100gm./min.		O$_2$/G in mM/mM		Cerebral R.Q.	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Thirteen Normal Subjects																				
M. G. L.	87	83	2	2	73	83	1.2	1.0	6.46	5.97	4.7	4.9	—	—	—	—	—	—	0.97	0.94
W. W.	92	96	8	—	78	65	1.1	—	6.99	6.58	5.4	4.3	—	—	—	—	—	—	0.99	0.95
Rb. M.	80	86	—	—	81	71	—	—	5.67	5.56	4.6	3.9	—	—	—	—	—	—	0.92	0.90
A. R.	78	82	4	6	63	61	1.2	1.2	6.53	6.32	4.1	3.9	—	—	—	—	—	—	0.85	0.97
J. K.	76	75	4	6	66	55	1.1	1.3	4.79	4.22	3.1	2.3	5.4	4.8	3.5	2.7	7.1	7.0	0.99	1.02
D. B.	93	97	7	7	53	47	1.6	1.9	7.65	7.05	4.0	3.3	7.3	8.7	3.9	4.1	8.4	6.5	0.93	1.03
M. L.	92	95	8	6	58	64	1.4	1.4	6.75	6.99	3.9	4.5	6.3	8.3	3.7	5.3	6.8	8.6	0.90	0.95
L. N.	90	94	3	2	67	62	1.3	1.5	5.57	6.30	3.7	3.9	8.6	8.7	5.7	5.4	5.2	5.8	0.84	0.95
H. H.	85	109	8	5	54	43	1.4	2.4	6.09	9.99	3.3	4.3	12.8	14.6	7.0	6.2	3.8	5.5	0.91	0.99
C. S.	81	96	7	9	64	87	1.2	1.0	5.36	4.91	3.4	4.3	9.8	8.5	6.3	7.4	4.4	4.6	1.02	0.93
Rn. M.	98	105	7	7	—	—	—	—	5.42	5.26	—	—	10.0	6.2	—	—	4.4	6.8	0.87	0.81
D. H.	87	86	5	5	75	93	1.1	0.9	3.63	3.23	2.7	3.0	—	—	—	—	—	—	1.16	1.32
M. G.	78	77	5	10	72	88	1.0	0.8	5.37	5.51	3.9	4.8	11.9	—	8.6	—	3.6	—	0.95	0.98
Mean	85.9	90.8	5.7	5.9	67.0	68.2	1.24	1.34	5.87	5.99	3.90	3.95	9.0	8.5	5.5	5.2	5.5	6.4	0.95	0.98
Standard error	±1.9	±2.9	±0.6	±0.8	±2.6	±4.7	±0.05	±0.16	±0.29	±0.45	±0.22	±0.22	±0.9	±1.2	±0.7	±0.7	±0.6	±0.5	±0.02	±0.03
p*	<0.05		>0.5		~0.9		>0.4		>0.7		>0.8		>0.9		~0.7		~0.3		>0.1	
Nine Schizophrenic Patients																				
V. S.	93	99	7	9	68	65	1.3	1.4	5.92	6.54	4.0	4.3	—	—	—	—	—	—	0.97	0.88
T H.	86	95	5	8	86	96	0.9	0.9	4.73	3.95	4.0	3.8	7.7	5.9	6.6	5.7	4.9	5.4	0.91	1.28
R. T.	88	110	12	13	75	75	1.0	1.3	5.68	5.92	4.2	4.4	5.1	7.7	3.8	5.8	9.0	6.2	1.01	1.01
P. L.	90	91	8	8	68	51	1.2	1.6	5.32	7.40	3.6	3.8	5.0	13.0	3.4	6.6	8.5	4.7	0.90	0.92
J. W.	85	91	7	8	75	79	1.0	1.1	6.68	5.39	5.0	4.3	9.0	9.7	6.8	7.7	5.9	4.5	1.09	0.88
W. H.	85	90	6	6	86	80	0.9	1.1	5.03	4.37	4.3	3.5	—	—	—	—	—	—	0.94	1.04
W. C.	91	89	9	10	66	69	1.3	1.1	5.95	5.47	3.9	3.8	8.7	8.6	5.7	5.9	5.5	5.1	0.95	0.90
B. G.	99	109	4	7	52	—	1.8	—	6.09	6.29	3.2	—	10.7	10.1	5.5	—	4.6	5.0	0.90	0.99
D. L.	97	99	9	9	72	87	1.2	1.0	4.78	4.52	3.4	3.9	7.9	9.2	5.7	8.0	4.9	4.0	0.90	1.06
Mean	90.4	97.0	7.4	8.7	72.0	75.2	1.18	1.19	5.58	5.54	3.96	3.98	7.7	9.2	5.4	6.6	6.2	5.0	0.95	1.00
Standard error	±1.7	±2.7	±0.8	±0.7	±3.5	±4.9	±0.09	±0.08	±0.22	±0.38	±0.18	±0.11	±0.8	±0.8	±0.5	±0.4	±0.7	±0.3	±0.02	±0.04
p*	~0.02		~0.02		>0.8		~0.3		>0.9		>0.6		>0.2		<0.1 > 0.05		<0.1 > 0.05		>0.4	

I: control.
II: during period of LSD-25 effect.
* Determined by the method of paired comparison.

TABLE 3

EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON BLOOD CONSTITUENTS

Subject	Art. hemoglobin concentration in grams %		Blood O₂ content in volumes %				Blood CO₂ content in volumes %				Blood glucose concentration in mgs. %				Blood pH				Blood CO₂ tension in mm. Hg				Blood O₂ saturation in percentage			
			Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Thirteen Normal Subjects																										
M. G. L.	16.49	16.25	20.66	20.81	14.20	14.84	45.63	45.38	51.91	50.96	—	—	—	—	7.35	7.35	7.33	7.29	44	44	52	54	92.5	93.2	69.1	65.1
W. W.	14.07	15.09	18.75	20.12	11.76	13.54	47.94	46.58	54.68	52.85	—	—	—	—	7.41	7.39	7.36	7.36	40	42	50	49	96.7	96.4	62.9	66.5
Rb. M.	15.18	15.63	19.14	19.15	13.47	13.59	48.26	46.28	53.45	51.27	—	—	—	—	7.42	7.40	7.40	7.33	40	40	46	50	96.8	93.6	72.2	70.9
A. R.	14.68	15.17	19.50	20.10	12.97	13.78	49.74	48.57	55.30	54.69	—	—	—	—	7.42	7.44	7.37	7.40	41	38	49	47	96.3	96.1	66.5	69.1
J. K.	13.57	13.98	18.12	17.68	13.33	13.46	46.26	45.91	51.01	50.22	84.5	74.4	79.1	69.6	7.43	7.39	7.35	7.35	37	40	47	47	98.0	95.7	70.3	72.5
D. B.	15.09	15.63	20.65	21.20	13.00	14.15	41.34	38.04	48.46	45.27	107.8	109.7	100.5	101.0	7.47	7.46	7.41	7.43	35	30	41	37	98.1	98.5	63.8	67.3
M. L.	13.49	14.35	18.08	18.42	11.33	11.43	49.55	47.97	55.64	53.58	82.3	86.3	76.0	78.0	7.38	7.37	7.35	7.34	44	43	51	51	99.1	97.6	63.0	60.4
L. N.	12.38	13.49	16.91	17.72	11.34	11.42	46.85	44.70	51.53	50.66	98.7	99.3	66.7	64.2	7.43	7.42	7.40	7.35	37	37	42	46	98.7	99.3	66.7	64.2
H. H.	15.63	16.70	20.46	22.16	14.39	12.17	48.89	30.02	54.44	39.94	93.7	129.4	80.9	114.8	7.45	7.54	7.37	7.45	39	20	50	51	98.4	99.8	67.9	54.4
C. S.	12.46	13.66	16.68	17.72	11.32	12.81	45.97	46.13	51.46	50.69	96.8	92.7	87.0	84.2	7.39	7.35	7.34	7.31	39	43	48	50	99.8	99.8	65.7	70.3
Rn. M.	14.89	15.63	19.70	20.49	14.28	15.23	46.96	45.28	51.67	49.55	89.5	91.0	79.5	84.8	7.39	7.37	7.36	7.34	40	41	48	49	99.0	98.8	70.5	72.2
D. H.	13.37	13.12	17.53	17.47	13.90	14.24	45.06	44.56	49.28	44.81	—	—	—	—	7.38	7.38	7.35	7.33	40	39	45	47	99.0	97.9	77.0	77.6
M. G.	12 91	12.91	17.03	17.14	11.66	11.63	46.31	45.20	51.43	50.59	101.0	94.9	89.1	—	7.39	7.40	7.34	7.35	40	38	48	47	98.0	97.0	66.7	65.1
Mean	14.17	14.74	18.71	19.25	12.84	13.25	46.83	44.20	52.34	49.62	94.3	97.2	82.4	85.2	7.41	7.40	7.36	7.36	39.7	38.1	47.5	48.1	97.7	97.2	67.9	67.4
Standard error	±0.35	±0.34	±0.40	±0.46	±0.34	±0.35	±0.62	±1.37	±0.62	±1.12	± 3.0	±5.8	±3.5	±6.7	±0.01	±0.01	±0.01	±0.01	±0.7	±1.8	±0.9	±1.1	±0.5	±0.6	±1.1	±1.6
p*	<0.01		<0.01		>0.1		$<0.1 > 0.05$		<0.02		>0.5		~ 0.5		>0.6		>0.4		>0.3		>0.3		>0.1		~ 0.7	
Nine Schizophrenic Patients																										
V. S.	11.25	13.37	17.00	18.10	11.08	11.56	46.40	44.02	52.15	49.79	—	—	—	—	7.48	7.48	7.46	7.43	32	32	37	39	96.9	94.9	66.2	66.3
T. H.	13.66	14.35	18.15	18.76	13.42	14.81	47.32	46.30	51.63	51.37	72.7	75.8	65.0	69.9	7.38	7.37	7.32	7.34	42	42	51	54	97.2	95.5	70.0	73.9
R. T	14.77	16.25	19.79	21.50	14.11	15.58	49.38	46.38	55.13	52.33	83.7	83.4	78.6	75.7	7.36	7.40	7.30	7.34	46	41	57	51	97.4	98.8	67.3	70.3
P. L.	14.77	15.18	19.05	19.42	13.73	12.02	43.15	36.61	47.96	43.44	173.0	155.0	168.0	142.0	7.49	7.46	7.34	7.42	32	28	44	36	98.4	97.9	68.8	58.2
J. W.	14.35	14.48	18.29	18.40	11.61	13.01	42.70	44.58	49.98	49.31	101.0	97.7	92.0	88.0	7.41	7.38	7.33	7.34	36	40	49	48	98.0	97.5	59.6	66.8
W. H.	13.28	13.57	18.01	17.88	12.98	13.51	48.27	48.94	52.01	53.48	—	—	—	—	7.41	7.41	7.37	7.37	40	39	46	48	98.0	97.6	69.0	72.0
W. C.	13.20	13.57	17.75	17.20	11.80	11.73	48.86	46.67	54.50	51.58	87.7	98.3	79.0	89.7	7.37	7.37	7.32	7.31	43	42	54	52	99.4	97.2	71.5	71.1
B. G.	15.65	16.49	20.14	21.53	14.05	15.24	42.68	36.31	48.19	42.56	96.6	119.0	85.9	108.9	7.40	7.38	7.34	7.33	38	34	47	43	97.8	98.0	67.3	67.0
D. L.	13.57	14.15	18.50	18.52	13.72	14.00	48.02	46.19	52.32	50.98	91.5	94.6	83.6	85.4	7.37	7.37	7.33	7.32	43	42	51	51	97.9	97.7	71.9	72.7
Mean	13.83	14.60	18.52	19.03	12.94	13.50	46.31	44.00	51.54	49.43	100.9	103.4	93.2	94.2	7.41	7.40	7.35	7.36	39.1	37.8	48.4	46.9	97.9	97.2	68.0	68.8
Standard error	±0.42	±0.38	±0.33	±0.51	±0.38	±0.51	±0.91	±1.50	±0.83	±1.29	±12.7	±10.0	±12.9	±9.2	±0.02	±0.01	±0.02	±0.01	±1.7	±1.7	±2.0	±2.1	±0.2	±0.4	±1.2	±1.6
p*	<0.01		$<0.1 > 0.05$		>0.1		<0.05		~ 0.02		>0.6		>0.8		>0.4		>0.3		>0.1		>0.1		>0.1		>0.6	

I: control.
II: during period of LSD-25 effect.
* Determined by the method of paired comparison.

Arterial hemoglobin concentration was significantly elevated by LSD-25 in both normal subjects and schizophrenic patients, rising from mean control values of 14.17 and 13.83 gm. per cent to mean experimental values of 14.74 and 14.60 gm. per cent, respectively ($p < 0.01$ in both groups). As a consequence of the hemoconcentration, mean arterial oxygen content rose from 18.71 to 19.25 vol. per cent ($p < 0.01$) in the normal group, but in the schizophrenic patients the increase to 19.03 vol. per cent during the LSD-25 action from a control value of 18.52 vol. per cent only approached statistical significance ($p < 0.1 > 0.05$). The slight increases in oxygen content observed in the internal jugular venous blood were not statistically significant. Arterial carbon dioxide content was reduced in normal subjects by LSD-25 from a mean control value of 46.83 vol. per cent to 44.20 vol. per cent, a change barely lacking statistical significance ($p < 0.1 > 0.05$). Internal jugular venous carbon dioxide content was reduced significantly from 52.34 to 49.62 vol. per cent ($p < 0.02$). In the schizophrenic patients LSD-25 caused a fall in both arterial and cerebral venous carbon dioxide contents, the former decreasing from 46.31 to 44.00 vol. per cent ($p < 0.05$) and the latter from 51.54 to 49.43 vol. per cent ($p \sim 0.02$). It is likely that the tendency for carbon dioxide contents of the arterial and cerebral venous bloods to fall during LSD-25 action was the result of hyperventilation. The fact that the decreases in carbon dioxide tensions in the same bloods were not statistically significant probably reflects only the greater precision of the method for measuring blood carbon dioxide content as compared with that for calculating blood carbon dioxide tension. In both the arterial and internal jugular venous bloods of both the normal subjects and the schizophrenic patients, glucose concentration, *p*H, and the percentage of oxygen saturation of hemoglobin remained unchanged by LSD-25.

Discussion

Perhaps the most obvious and striking feature of the results reported here is the remarkable paucity of effects of LSD-25 on the cerebral circulatory and metabolic functions studied. Indeed, except for the minimal increases in arterial hemoglobin concentration and mean arterial blood pressure and a marked dilatation of the pupils, there is little in the results of these physiological studies to indicate any activity on the part of the drug. This is equally true for both the normal and schizophrenic groups. On the other hand, the results of the psychological and psychiatric evaluations of the drug action clearly demonstrated the characteristic responses ascribed to LSD-25. It is, therefore, necessary to conclude that the disturbances in psychological and mental functions produced by lysergic acid are unrelated to any changes in the cerebral circulation or those aspects of the metabolism that are reflected in the rates of oxygen and glucose utilization by the brain as a whole.

These results concerning the effects of LSD-25 on the *in vivo* cerebral metabolism are in contrast with some of those obtained in studies on cerebral tissue *in vitro*. For example, Mayer-Gross, McAdam, and Walker[9] have reported that in guinea pig brain brei, lysergic acid produced a stimulation of oxygen consumption associated with a decreased utilization of hexosemonophosphate.

Grenell[10] has observed a similar stimulation of oxygen consumption by LSD-25 in cortical homogenates but one associated with a decrease in the recoverable adenosinetriphosphate. On the other hand, Lewis and McIlwain[11] have observed slight depressions in oxygen consumption produced by LSD-25 in cortical slices of guinea pig brain, and the inhibitory effect of the drug was enhanced by electrical stimulation of the tissue slices. Geronimus and his co-workers[12] have also observed an inhibitory effect of lysergic acid on brain oxygen consumption *in vitro*. In view of these contradictory findings *in vitro*, which are in disagreement not only with our *in vivo* findings but also among themselves, it is difficult to evaluate their significance. Indeed, in an organ such as the brain in which the normal function of the cellular elements depends to so great an extent on the normal interaction of those elements, particularly as regards the processes affected by LSD-25, any effects observed *in vitro* that are in disagreement with those *in vivo* must be seriously questioned before attaching any great functional significance to them.

It is possible that the action of lysergic acid is associated with changes in cerebral circulation or metabolism, but in areas representing so small a fraction of the total brain that the effects are obscured in measurements in the brain as a whole. Alternatively, it may be that in a heterogeneous organ like the brain, many of whose parts are functionally inversely or reciprocally related, changes in metabolic activity in some areas are balanced by inverse changes in other areas so that the net metabolic rate of the brain remains unchanged. These possibilities await further evaluation by methods that measure blood flow and metabolism in localized areas of the brain *in vivo*.

The psychotomimetic state produced by lysergic acid must now be added to a growing list of conditions in which gross alterations in mental and psychological functions are not related to any significant changes in cerebral circulation or metabolic rate. Thus a similar lack of changes in the latter functions has been observed in schizophrenia,[21] during the performance of mental arithmetic,[22] and during the action of the tranquilizing drug, chlorpromazine.[23, 24] Also, although there is a slight but significant rise in cerebral blood flow, no change in cerebral metabolic rate occurs during natural sleep.[25] On the basis of the results obtained in studies such as these, it has become increasingly obvious that the cerebral processes underlying many mental and psychological functions are too subtle to reflect their changes in the over-all circulation and metabolism of the brain.

The only truly positive physiological changes observed in the present study were a marked mydriasis, a slight increase in mean arterial blood pressure, and a mild hemoconcentration. Whether the hemoconcentration represents an absolute increase in circulating hemoglobin mobilized from stored pools of red cells or is the result of a relative increase in hemoglobin concentration because of a loss of plasma volume remains undetermined. In any case, a similar combination of changes has been found to occur during the infusion of *l*-norepinephrine in man.[26] This similarity of effects between LSD-25 and *l*-norepinephrine may be of interest in view of the suggestion by Rinkel and his associates[27] that the action of lysergic acid may be related to its interference somewhere in the adrenalin cycle. Although it must be pointed out that the elevated blood pressure and the hemoconcentration could both be explained by the increased motor activity that our subjects and patients exhibited under the influence of LSD-25, these findings are not inconsistent with the concept that LSD-25 exerts its effect through some involvement in the epinephrine system.

Summary

(1) Studies of the cerebral circulation and metabolism during a control period and during the height of action of intravenously administered LSD-25 were performed in 13 normal or nonpsychotic subjects and 9 schizophrenic patients.

(2) Despite the occurrence of the characteristic psychological and mental effects of LSD-25, there were no changes produced by the drug in cerebral blood flow, vascular resistance, oxygen and glucose utilization, or R.Q. in either the nonpsychotic or schizophrenic subjects.

(3) Except for mydriasis, a slight elevation in mean arterial blood pressure, and a moderate increase in arterial hemoglobin concentration, the latter associated with a comparable rise in blood-oxygen content, LSD-25 did not produce any changes of significance in the various physiological functions and blood chemical constituents studied in both groups of subjects.

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Part II. Psychotomimetic Agents: Neurophysiological Effects

CHAIRMAN'S INTRODUCTION

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The present growth of interest in biological studies of mental disease has been catalyzed by 2 discoveries: the ameliorating effect of reserpine and chlorpromazine in schizophrenia, and the discovery of psychotomimetic drugs that have minimal side effects. Thus any scientist who can spare 8 hours from his busy life and who has a good friend to act as his keeper can enjoy the fascinating revelations of temporary insanity. Your chairman has experienced the effect of *d*-lysergic acid diethylamide (LSD-25) in effective doses 10 or more times, and assuredly the best friend of the scientist during periods of psychosis induced by LSD is the clock that ticks off the minutes and hours that result ultimately in the reversal of the effect of the drug and the return of the subject to normal neurotic behavior.

In times past, the organic chemist has not been successful in obtaining tests in normal man of the drugs that affect behavior. Roger Adams, for instance, about 20 years ago had derivatives of marihuana tested by the British scientist G. T. A. Stocking. Before 1940, only a few psychologists such as Heinrich Klüver and anthropologists such as Adamson Hoebel had partaken of mescaline or chewed the peyote cactus. Now all has changed! Medical students, journalists, and television announcers clamor for the chance to be "lysergized" in order to tell their friends and to sell to the world the stories of their trips to the brink of insanity. This "brinkmanship," this ubiquitous interest in insanity, may eventually prove to be a help in relieving the ever-growing burden of our present predominantly custodial care of the mentally ill.

This section of this monograph will teach us more about basic neuropharmacological observations on the possible mechanisms of the action of psychotomimetic drugs.

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A REVIEW OF THE NEUROPHYSIOLOGICAL EFFECTS OF LYSERGIC ACID DIETHYLAMIDE (LSD) AND OTHER PSYCHOTOMIMETIC AGENTS

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Introduction

Knowledge of the psychological effects of psychotomimetic agents has been available for a long time. Reports concerning the effects of marihuana extend back over many centuries.[1, 2] It is only recently, however, that studies of the electrophysiological effects of these agents have been undertaken. The relative lack of data concerning the electrophysiological effects of psychotomimetic agents is only partially the result of the recent origin of electrophysiological techniques. Even within recent years neuropharmacologists have tended to ignore drugs whose effects were primarily psychotomimetic. Certain psychotomimetic agents such as cocaine,[3, 4, 5] that have somatic as well as psychological effects, have been subjected to intensive neurophysiological investigation. The bulk of this investigation, however, has been directed to the elucidation of mechanisms underlying the somatic effects of cocaine. There have been many studies of the effects of cocaine on excitation and conduction in the peripheral nerves, while relatively little attention has been devoted to the neural processes involved in cocaine-induced hallucinations.

Before presenting data concerning the electrophysiological effects of psychotomimetic agents, it is necessary to decide upon a definition of the class of agents to be considered. In a recent paper on drug-induced psychoses, Hoch, Pennes, and Cattell[6] have presented a partial list of drugs that may produce psychotic reactions. This list includes barbiturates, bromides, chloral hydrate, amphetamine, caffeine, aspirin, acetanilid, atropine, scopolamine, diisopropyl fluorophosphate (DFP), cocaine, quinine, Atabrine, ergot alkaloids, lead, arsenic, mercury, various hormones, and even water in toxic amounts. A complete list of agents that produce psychoses would be considerably longer. If all of these agents were to be included, the group of psychotomimetic agents would be extremely heterogeneous. As Hoch, Pennes, and Cattell[6] point out, however, the psychopathological characteristics of drug-induced psychoses allow the psychotogenic drugs to be divided into at least 2 categories. Drugs within the first category produce a psychological disturbance similar to that seen in the organic psychoses. The second category of drugs produces a qualitatively different psychological disorder, one that may be likened to the functional psychoses. The term psychotomimetic refers to those agents that produce the second type of psychological disorder. These 2 classes of drugs differ in the degree to which they alter orientation, memory, and awareness. Both classes of drugs may produce mood alterations, hallucinations, distortions of the body image, and a wide variety of additional psychological effects, but the psychotomimetic drugs produce these psychological changes without causing the gross impairment of memory and orientation that is characteristic of the organic psychoses. The statement that psychotomimetic agents produce psychoses that are qualitatively different from the organic psychoses should be qualified, since there may be organic psychoses with relatively little impairment of memory and awareness, while "functional" psychoses may at times be marked by gross disorders of memory, orientation, and awareness. Indeed, it might well be argued that the psychotomimetic agents cause a disorder that lies on a continuum with the organic psychoses, differing from them only in certain limited quantitative respects. For the purposes of the present review, however, the analogy between "functional" psychoses and effects of psychotomimetic agents will be accepted. The definition of psychotomimetic agent also should specify that the psychosis must occur in a high proportion of subjects to whom the agent is administered. In essence, then, a psychotomimetic agent is one that reliably causes a psychopathological syndrome similar to that seen in the functional psychoses. If this definition be applied, psychotogenic substances such as adrenocorticotropic hormone (ACTH),[7-9] cortisone,[10-12] and isopropylisonicotinyl hydrazine[13, 14] would not be called psychotomimetic agents, since psychoses do not occur with sufficient frequency following their administration.

A considerable number of chemical substances may be classified as psychotomimetic agents even within the restricted definition that has been suggested. Among these agents are: lysergic acid diethylamide (LSD),[15] mescaline,[16] adrenochrome,[17] harmine,[18] tetrahydrocannabinol,[1] DFP,[19, 20] tetraethylpyrophosphate (TEPP),[21] and *N*-allylnormorphine.[22, 23] Yohimbine has been said to be a psychotomimetic agent,[24] but studies of its effects generally have failed to demonstrate any psychotomimetic properties of the drug.[25-28] Fabing and Hawkins[29] have reported that large intravenous doses (8 to 16 mg.) of 5-hydroxy-3(β-dimethylaminoethyl) indole (bufotenin) may have psychotomimetic effects. Isbell[30] has also observed striking psychological changes following the intramuscular administration of similar quantities of the drug.

Of the various psychotomimetic agents that have been listed, several will be omitted in the subsequent review of the electrophysiological effects of agents of this class. DFP and other anticholinesterases will not be considered, since extensive reviews of the electrophysiological effects of acetylcholine and cholinesterase inhibitors[31-33] already exist. Other agents, such as harmine, have not been included because of the lack of studies of their electrophysiological effects. Agents that will be included in the review to follow are LSD, mescaline, adrenochrome, marihuana, tetrahydrocannabinol, and *N*-allylnormorphine.

The electrophysiological effects of psychotomimetic agents may, for descriptive purposes, be divided into 2 general classes: (1) effects on transmission in specific synaptic systems and (2) effects on spontaneous cerebral activity. The studies dealing with the effects of psychotomimetic agents on specific synaptic systems will be described first.

Effects of Psychotomimetic Agents on Synaptic Transmission

(1) *LSD.* Marrazzi and Hart[34, 35] have studied the effects of LSD on the cortical response to stimulation of the corresponding area of the contralateral cerebral hemisphere. These workers found that intracarotid administration of 8 μg./kg. of LSD in a cat treated with pentobarbital sodium reduced the amplitude of the postsynaptic component of the transcallosal response. Purpura[36, 37] has obtained similar results in unanesthetized cats.

Purpura[36, 37] has studied the effects of LSD on evoked potentials in the auditory and visual systems. He found that LSD caused facilitation of the primary cortical responses to auditory (click) and visual (photic) stimulation in cats immobilized with succinylcholine. In addition, this investigator found that LSD in intravenous doses as low as 4 μg./kg. altered the recovery cycles in these 2 systems. The alterations of the recovery cycle observed by Purpura consisted of a shortened initial recovery phase and a prolonged phase of supernormality. Higher doses of LSD, from 40 to 60 μg./kg., caused depression of the auditory evoked potential while there was still facilitation of the potentials evoked by photic stimulation. The facilitation of evoked cortical potentials was not seen in animals prepared with pentobarbital sodium in which, at high doses of LSD, inhibition of the evoked auditory and visual potentials occurred regularly. Purpura found that the cortical response to lateral geniculate radiation shock was also facilitated by LSD. He made simultaneous observations on the effects of LSD on the response of the visual cortex to stimulation of geniculate radiation fibers and of the homolateral suprasylvian gyrus. LSD (30 μg./kg.) caused inhibition of the striate response to suprasylvian stimulation while simultaneously causing facilitation of the striate response to geniculate radiation stimulation. The facilitatory effect of suprasylvian stimuli on the response to subsequent geniculate radiation stimulation was blocked by 15 μg./kg. of LSD. These data led Purpura to conclude that LSD exerts differential effects on axodendritic as compared to axosomatic synapses, inhibiting axodendritic transmission while causing facilitation of axosomatic transmission.

The effects of LSD on the cortical response to photic stimulation have been studied by Rovetta[38] in anesthetized cats. A topical application of LSD in concentrations up to 9 per cent was without clear effect on the cortical response, and intravenous administration of 40 μg./kg. of LSD was also without effect on the cortical response.

Evarts *et al.*[39] studied the effects of LSD on synaptic transmission in the visual system of the cat. In cats prepared with pentobarbital sodium, intracarotid administration of 30 μg./kg. of LSD caused a mean decrease of 80 per cent in the amplitude of the geniculate postsynaptic response to a single shock to the optic nerve. Whereas geniculate transmission was blocked by LSD, transmission within the retina and between geniculate radiation fibers and cortical cells was extremely resistant to inhibition by LSD.[39] Onset of the effect on geniculate transmission was rapid, occurring within 5 to 10 seconds of the administration of LSD, and recovery occurred within 1 hour. With intravenous administration, a greater amount of LSD was required to produce a comparable effect, 160 μg./kg. of LSD producing an average decrease of 60 per cent in the amplitude of the geniculate postsynaptic response. Pentobarbital sodium was found to sensitize the preparations to LSD, and studies carried out in conscious cats with implanted electrodes[40] have shown that the amount of LSD required to block geniculate transmission of a single optic-nerve volley is approximately 5 times greater in the conscious cat than in the cat prepared with pentobarbital sodium. Simultaneous observations of behavioral and electrophysiological responses in cats with chronically implanted electrodes showed that a block of geniculate transmission in the cat was associated with an absence of responsiveness to visual stimuli. Throughout the period of behavioral blindness, however, the cats continued to respond briskly to auditory stimuli, orienting to the source of the sound. The occurrence of transient blindness following high doses of LSD (0.5 to 1.0 mg./kg.) has also been found to occur in the monkey.[41]

The studies cited in the preceding paragraphs have demonstrated marked differences in the sensitivity of various synapses to the blocking effects of LSD. Among the most sensitive synapses are those involved in the transcallosal and suprasylvian-striate reactions. The lateral geniculate synapse is less sensitive, whereas the synapses of the retina and those between lateral geniculate radiations and cells in the visual cortex are extremely resistant to the blocking effects of LSD.

Preliminary data on the nature of the change in geniculate excitability induced by LSD have been provided by the observations of Evarts *et al.*,[39] who found that marked recruitment occurred during depression of the geniculate postsynaptic response. The excitability cycle obtained with paired stimuli to the optic nerve was similar in subnormality induced by LSD and in subnormality induced by brief conditioning trains.[42] It was also found that the blocking effects of LSD on geniculate transmission were overcome transiently by asphyxia[39] and by prolonged tetanization of the optic nerve.[43] The effects of tetanization of the optic nerve on the block of geniculate transmission induced by LSD are reminiscent of the phenomenon of posttetanic decurarization in the skeletal musculature.[44, 45]

Killam and Killam[46] have studied the effects of LSD on the thalamic recovery cycle by recording the thalamic activity evoked by peripheral stimulation of mixed nerves in the unanesthetized cat treated with curare. They found that LSD in quantities up to 100 μg./kg. altered neither the thalamic recovery cycle nor the amplitude of the recorded electrical activity. These results are in contrast to the results of Evarts *et al.*,[39] who found that LSD blocked geniculate transmission. This apparent difference, however, is probably based on the differences in operational procedures and, possibly, on the different sensory pathways that were investigated. The thalamic activity recorded by Killam and Killam[46] in response to stimulation of peripheral nerves is characteristically spread out in time, making it impossible to separate presynaptic and postsynaptic components of the thalamic response. Moreover, animals that have been treated with curare, like conscious unrestrained animals, are considerably more resistant to the effects of LSD on geniculate transmission than are animals under the influence of pentobarbital sodium. FIGURE 1 shows the effects of successive intravenous doses of LSD on geniculate transmission in a cat that

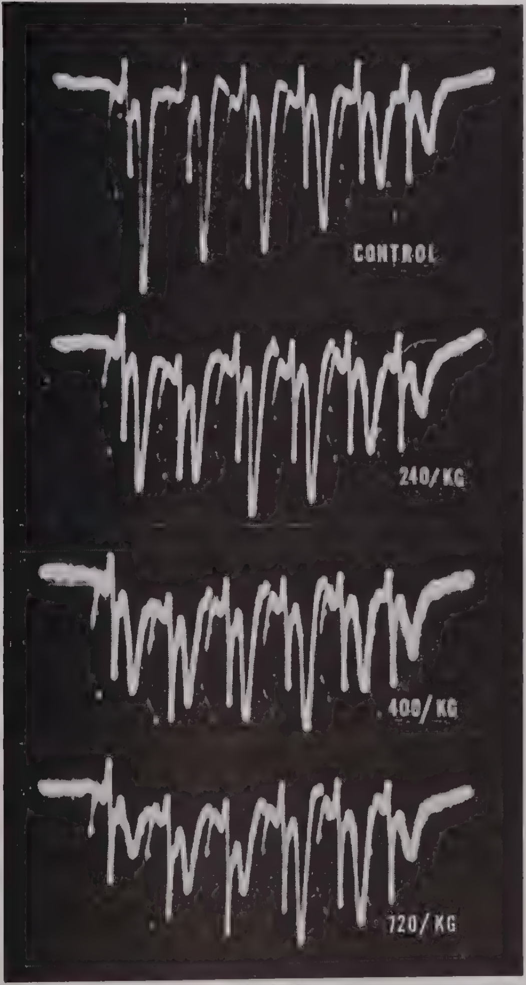

FIGURE 1. The effects of lysergic acid diethylamide (LSD) on geniculate transmission in the unanesthetized cat treated with curare. The control tracing shows the geniculate response to a tetanus of 6 shocks applied to the contralateral optic nerve. The interval between successive shocks is 2.5 msec. The effects of LSD in cumulative dosages of 240, 400, and 720 μg./kg. intravenously are shown. Note the marked decrease in the geniculate postsynaptic response and the absence of alteration of amplitude of the presynaptic tract spike.

had received curare. A clear decrease in the amplitude of the geniculate response was seen after 240 μg./kg. A marked decrease was present when the animal had received a total dose of 720 μg./kg. At this level of depression there was marked recruitment. The experimental results of Evarts *et al.*[39] and of Killam and Killam[46] do not, therefore, appear contradictory. Both groups of experiments on thalamic transmission in unanesthetized preparations show no effect of LSD in doses of less than 100 μg./kg.

Slater *et al.*[47] have studied the effects of close intra-arterial injections of serotonin and LSD on the flexion reflex in the cat. They found that serotonin caused facilitation followed by depression, and that LSD blocked these effects of serotonin. The possibility that the vascular effects of serotonin were of significance in this effect remains to be ruled out.

(2) *Mescaline.* Hart, Langfitt, and Marrazzi[48] and Hart and Marrazzi[49, 50] have found that mescaline inhibits the postsynaptic component of the transcallosal response. The effects of mescaline on the transcallosal response were prevented by chlorpromazine and azacyclonol.[48] Rovetta[38] found that topical application of mescaline chloride to the optic cortex in anesthetized cats caused a marked increase in the cortical response to auditory and photic stimulation and to local cortical stimulation. Local application of mescaline to the lateral geniculate caused an increase in the geniculate response to photic stimulation. Rovetta found that "strychnine and mescaline acted identically on evoked responses to light flash." In contrast to these effects of topically applied mescaline, intravenous mescaline in doses of the order of 20 mg./kg. caused a slight and variable (0 to 20 per cent) decrease in the amplitude of both cortical and geniculate responses. Pennes[51] has studied the effects of mescaline on cortical potentials evoked by photic stimulation and by electrical stimulation of the optic nerve. The results of his studies were contradictory to those of Rovetta.[38] Pennes found that topical application of mescaline to the optic cortex had no constant effects on photically or electrically evoked potentials, whereas intravenous administration of mescaline (5 to 10 mg./kg.) reduced or eliminated the evoked potentials. The explanation of the differences between the results of Rovetta and those of Pennes is not readily apparent. Rovetta's experiments were carried out in cats anesthetized with chloralose-urethane, and Pennes' observations were made in cats anesthetized with pentobarbital sodium, chloralosane, or chloralosane urethane.

The Effects of Psychotomimetic Agents on Spontaneous Cerebral Activity

(1) *Lysergic acid diethylamide (LSD).* Studies of the effects of LSD on spontaneous cortical activity in animals have been reported by a number of investigators. Delay,[52] and Delay, Lhermitte, and Verdeaux[53] found that LSD caused a decrease in the amplitude of spontaneous cortical activity in rabbits, but that the substance did not block the electroencephalographic (EEG) response to activation. Bradley[54] and Bradley and Elkes[55] have studied the effects of LSD on the EEG of several different types of cat preparations. In the conscious, unrestrained cat from which electrical activity was recorded with implanted electrodes, LSD (from 15 to 25 μg./kg. by mouth) was followed by an EEG pattern which was not unlike that seen in the normal alert animal, and that consisted of diffuse, low-amplitude, fast (from 15 to 30 counts per second) activity. The EEG of cats that had not received LSD showed higher amplitude and slower activity. These authors found that LSD in amounts up to 100 μg./kg. was without effect on the spontaneous electrical activity recorded from spinal (*encéphale isolé*) or decerebrate (*cerveau isolé*) animals. These findings led Bradley[54] and Bradley and Elkes[55] to conclude that the effects of LSD on the corticogram depend on the presence of spinal connections.

Schwarz *et al.*[56] studied the effects of LSD injected into the cerebral ventricle of the unanesthetized unrestrained cat. Intraventricular administration of 15 μg. of LSD was followed by low-amplitude fast activity associated with occasional slow waves and "consistent with a relaxed state."

Several investigators have studied the effects of LSD on the EEG of cats anesthetized with pentobarbital sodium (Nembutal). Under these conditions Bradley,[54] Bradley and Elkes,[55] and Evarts *et al.*[39] found that LSD abolishes the "barbiturate spindles" that are characteristic of the EEG of the animal under the influence of pentobarbital sodium.

Himwich[58] and Rinaldi and Himwich[57, 59] have studied the effects of LSD on the spontaneous cerebral electrical activity in rabbits treated with curare. These authors found that small doses of LSD (1 to 5 μg./kg.) caused a decrease in the amplitude and an increase in the frequency of the EEG, while higher doses of LSD (10 μg./kg.) caused a continuous "alert" pattern. Still higher doses (20 to 60 μg./kg.) caused the reappearance of slow waves. These effects of LSD were prevented by alpha-4-piperidyl benzhydrol HCl (azacyclonol). Although it prevented the "alerting" effects of LSD on the EEG, azacyclonol did not prevent the alerting effects of DFP or amphetamine. Rinaldi and Himwich[57, 59] concluded that one of the central effects of LSD was the stimulation of the "mesodiencephalic activating system." The effects of LSD on the threshold for EEG "arousal" have been studied by Killam and Killam[46] in cats treated with curare. These workers found that LSD in doses up to 100 μg./kg. administered intravenously caused slight increases in the threshold of arousal responses of the EEG to sciatic-nerve and reticular formation stimulation. These observations are in contrast to those of Rinaldi and Himwich,[59] who found that LSD (in doses of from 1 to 5 μg./kg.) caused a decrease in the threshold for the production of arousal of the EEG by stimulation of the reticular formation in the rabbit treated with curare.

The cortical-recruiting response that results from repetitive stimulation of anterior midline thalamic nuclei may be classed under the general category of EEG effects, although, of course, it does not represent spontaneous activity. Purpura[37] has found that LSD in doses from 10 to 30 μg./kg. depresses the recruiting response in cats immobilized with succinylcholine. Killam and Killam[46] obtained somewhat different results. Following LSD in doses of up to 100 μg./kg., recruiting responses were unchanged except for slight reductions in the amplitude of cortical responses. The differences in the results obtained by these investigators may be the result of the differences in the paralyzing agents used and also of the fact that Purpura's experiments on the recruiting response were carried out in animals that initially were anesthetized with barbiturates and then allowed to recover from anesthesia. Purpura[37] notes that the shortening of the period between the initial injection of pentobarbital sodium and the administration of LSD made it possible to produce more pronounced inhibition of the recruiting responses.

A number of studies of the effects of LSD on the EEG in man have been carried out. Rinkel *et al.*,[60] studying the effects of LSD on normal volunteers, found that LSD caused only slight changes in the EEG, these changes consisting principally of slight increases in the frequency of the alpha rhythm. Gastaut, Ferrer, and Castells,[61] also studying normal subjects, found that LSD caused an increase of the alpha frequency in 9 out of 10 subjects, the mean increase being 1.75 counts per second. Seven subjects showed an increase in photic driving, and 5 of these 7 showed irradiation of the photic response to the frontal region. Forrer and Goldner[62] did not observe any significant changes in the EEG in a large group of psychotic subjects who had received 2 μg./kg. LSD. Elkes, Elkes, and Bradley,[63] and Bradley, Elkes, and Elkes[64] studied the EEG effects of LSD in normal volunteers. The primary effect of LSD was an increase in the responsiveness of the alpha rhythm. Control EEG records of the "micro-alpha" type showed little change following administration of LSD. Records that were initially "responsive alpha" became more responsive, whereas "persistent alpha" records became "responsive alpha."

(2) *Mescaline.* The effects of mescaline on spontaneous electrical activity in the rabbit under curare have been studied by Himwich[58] and Rinaldi and Himwich,[59] who found that the effects of mescaline were almost identical to those of LSD. Mescaline in doses of 5 to 10 mg./kg., administered intravenously, caused an "alert" EEG pattern that could be restored to normal by 15 to 25 mg./kg. of azacyclonol. Schwarz *et al.*[56] found that the intraventricular administration of mescaline in conscious cats caused intermittent spikes and slow waves, together with a disturbance of behavior that consisted of crying, howling, and paroxysms of scratching.

Chweitzer, Geblewicz, and Liberson[65, 66] found that mescaline caused a decrease in the amplitude of alpha activity in man, with blocking of alpha activity during the occurrence of visual hallucinations. The decrease in alpha amplitude remained for several days, even after the disappearance of subjective symptoms. Rubin, Malamud, and Hope[67] studied the effects of mescaline on the EEG of psychotic patients. Doses of 0.3 gm. caused an increase in per cent of alpha in 4 of 7 patients and a decrease in per cent of alpha in the remaining 3 patients. There was no apparent correlation between the change in EEG and the subjective effects. Wikler[23, 68] found variable effects on the EEG in post-addicts following the administration of mescaline in oral doses of 5 mg./kg. The effects were as follows: (1) no change, (2) replacement of alpha activity by low-amplitude fast activity, or (3) an increase in the frequency of the alpha pattern. Denber and Merlis[69] studied the effects of mescaline on the EEG of schizophrenic patients. Following intravenous administration of 0.5 gm. of mescaline sulfate there was usually a decrease in the per cent of alpha. These authors did not observe changes in the EEG in association with verbal reports of visual hallucinations on the part of the subjects. Merlis and Hunter[70] found that mescaline caused a marked decrease in the amplitude of the delta activity present following a series of electroconvulsive treatments. Denber[71] found that delta activity in epileptics was also decreased by mescaline.

(3) N-*allylnormorphine.* The psychotomimetic effects of *N*-allylnormorphine have already been discussed.[22, 23] Studies of the effects of *N*-allylnormorphine on specific synaptic systems have not yet been carried out. Wikler and his co-workers have studied the effects of *N*-allylnormorphine on the EEG of conscious animals[72] and in man.[23, 68, 73] *N*-Allylnormorphine, 2.5 mg./kg. adminstered subcutaneously, in dogs that were conscious and not under curare

caused the appearance of a sleep pattern in the EEG, though the behavioral state of the animal remained alert. In man, 5 to 15 mg. of *N*-allylnormorphine produced a variety of changes in the EEG. In subjects who experienced relaxation and euphoria in response to the drug, the EEG changes consisted of an increase in rhythmicity and a slight decrease in the alpha frequency. The symptom of anxiety was associated with a decrease in the amplitude and rhythmicity of the EEG.

(4) *Adrenochrome.* Szatmari, Hoffer, and Schneider[74] have studied the effects of adrenochrome on the EEG of normal and epileptic subjects. These authors report that "A few volunteers received adrenochrome intravenously in 10–25 mg. dosage. Subjective changes were observed in only a few cases; these took the form of apprehension or foreboding expectations" and that "no specific change in the EEG pattern was observed." Whereas adrenochrome failed to produce changes in the EEG of normal subjects, this substance did have marked effects on the EEG in epileptic patients.[74] In these patients, intravenous doses of 10, 25, and 50 mg. of adrenochrome caused an increase of bilateral paroxysmal abnormalities. The apparent slight degree of subjective changes observed by these authors following 10 to 25 mg. of adrenochrome in normal subjects is in contrast to the marked psychological changes that were reported by Hoffer, Osmond, and Smythies[17] to result from administration of 5 mg. of adrenochrome.

Schwarz *et al.*[56] found that intraventricular administration of adrenochrome (1 mg.) in the conscious cat caused the appearance of 4-cycles-per-second slow waves, with low-voltage spike components. When the cats appeared to be aroused, however, the alert EEG pattern returned. Adrenolutin had effects that were generally similar to those of adrenochrome.

(5) *Marihuana and tetrahydrocannabinol.* The electroencephalographic effects of marihuana have been studied by Williams *et al.*[75] These authors found that the administration of 360 to 2400 mg./day of a synthetic pyrahexyl compound had variable effects on the EEG. There was a significant decrease in per cent alpha in 2 out of 5 subjects (postaddicts) and an increase in per cent alpha in 1 out of the 5. In one subject, persistent delta activity appeared during the period of drug administration. There did not appear to be any clear relationship between the EEG and the psychological effects of the drug. In another study, Wikler and Lloyd[76] found that there was little change in alpha frequency in subjects who smoked marihuana cigarettes, and in this group of subjects a decrease in per cent alpha occurred in about one half of the cases. Studies of the electrophysiological effects of tetrahydrocannabinol in lower animals have not been made.

The Interaction Between the Psychotomimetics and Other Pharmacological Agents

Hypotheses concerning the biochemical processes that constitute the mechanism of action of LSD and other psychotomimetic agents have been presented in this monograph. The present section of this review will not attempt to cover this area, but will seek merely to raise several points that are of particular interest in connection with the interaction of LSD and various other pharmacological agents in their effects on cerebral electrical activity. Interactions between serotonin and LSD have been described in a number of *in vitro* systems.[77-82] Because of the antagonism between LSD and serotonin *in vitro*, there has been considerable speculation that the psychological and electrophysiological effects of LSD *in vivo* are the result of serotonin-LSD interaction. The work of Shore, Silver, and Brodie[83] indicates that LSD also antagonizes certain effects of serotonin *in vivo*. It is therefore of interest that serotonin has failed to antagonize the electrophysiological effects of LSD in most of the *in vivo* systems in which the 2 substances have been studied. Marrazzi and Hart[34] found that LSD and serotonin had similar effects on the transcallosal potential, both agents causing inhibition. Schwarz *et al.*[56] found that intraventricular administration of serotonin did not alter the effects of previously or subsequently administered LSD. Evarts[84] did not observe any specific interaction between intracarotid LSD and serotonin with respect to actions on synaptic transmission in the lateral geniculate nucleus of the cat. Slater *et al.*[47] reported that the effects of serotonin on the flexion reflex were blocked by LSD. The converse of this observation, however, a reversal of an electrophysiological effect of LSD by serotonin, has not yet been demonstrated. Negative evidence, of course, is not conclusive; it is possible that the rapid destruction of serotonin prevents interaction between serotonin and LSD *in vivo*. The absence of psychotomimetic effects by 2-brom LSD,[85] however, and by a number of other *in vitro* serotonin antagonists makes it apparent that the hypothesis of LSD-serotonin interaction as the basis for the psychological effects of LSD is not adequate to explain the experimental observations now available.

Electrophysiological observations have also been employed as indicators of pharmacological interaction in studies of the mechanism of action of the "tranquilizing" drugs. One of these agents, azacyclonol,[86] has been reported to be an LSD antagonist *in vivo*. Fabing[87] found that azacyclonol blocked the development of the psychosis induced by LSD in man. Rinaldi and Himwich[69] found that azacyclonol blocked the effects both of mescaline and of LSD on the EEG in rabbits under the influence of curare. In an extensive and meticulously controlled study, however, Isbell[88] found that azacyclonol did not alter the psychological effects of LSD in man. Here, then, is an example of an agent that blocks the effects of LSD on the EEG in animals but that, according to the extensive studies of Isbell,[88] is without effect on the psychotomimetic properties of LSD in man. A similar failure of correspondence between electrophysiological and psychological interactions is seen in the case of reserpine and LSD. Purpura[57] has reported that "reserpine was shown to antagonize the action of LSD whether this action on the auditory primary was facilitatory, at low doses, or inhibitory, at higher doses." This antagonism by reserpine to an electrophysiological effect of LSD is in marked contrast to the observation of Isbell[89] that reserpine intensified the psychotomimetic effects of LSD in man. Hart, Langfitt, and Marrazzi[48] found that both azacyclonol and chlorpromazine antagonize the inhibitory effects of mescaline on transcallosal potentials. Chlorpromazine has been found by Bradley and Hance[90] to prevent the effects of LSD on the spontaneous cortical activity of the conscious cat. The correspondence between electrophysiological observations in animals and psychological observations in man is somewhat better in the case of chlorpromazine than it is with reserpine and azacyclonol. Hoch[91] has found that chlorpromazine decreases but does not prevent the psychotomimetic effects of LSD in man. Even in the case of chlorpromazine, however, the report of Bradley and Hance[90] that "chlorpromazine given either before or after administration of *R*-amphetamine or LSD-25 blocked the effects described for these drugs" would seem to predict a more striking interaction between chlorpromazine and LSD in man than has been found to be the case. It may be concluded that the interactions of psychotomimetic agents and tranquilizing drugs in animals do not allow reliable predictions of the interactions between the same psychotomimetic agents and tranquilizing drugs in man.

The Relationship Between the Electrophysiological and the Psychological Effects of Psychotomimetic Agents

The evaluation of the relationship between the electrophysiological effects of psychotomimetic agents and the psychological effects of these drugs is difficult, since such evaluation necessarily leads to a considerable amount of speculation that cannot be supported by experimental observations. The difficulty of establishing a causal relationship between electrophysiological and psychological events is, in fact, one of the factors that has discouraged many physiologists from undertaking studies of the actions of psychotomimetic agents. The effects of cocaine on the excitability of nerve fibers are generally accepted as being causally related to the local anesthesia that follows injections of cocaine in man. In the case of LSD, however, it does not appear that we have reached the point of being able to assign any particular psychological effect of the drug to a demonstrated disturbance of the electrical activity of the nervous system. Studies in animals have shown several effects of LSD at dosages that approach those that are used to produce experimental psychoses in man. Two of these sensitive indices of the effect of LSD in animals are the corticocortical responses that have been studied by Purpura[36, 37] and by Marrazzi and Hart.[34, 35] Both the transcallosal response[34] and the suprasylvian-striate reaction[37] are highly sensitive to the effects of LSD. Is it possible, then, that disturbance of corticocortical connections might be responsible for the disturbances of visual perception that result from the administration of LSD? At the present time it would not be possible to conclude that this is so. In the case of the callosal fibers, transmission may be interrupted permanently without any gross psychological effect. Akelaitis[92] has carried out a number of complete sections of the corpus callosum and the anterior commissure in man without apparent effect on the subjective or objective psychological processes. The mere interruption of callosal transmission, then, does not explain the effects of LSD on perception. What, then, might be the effects of such interruption of intrahemispheric corticocortical pathways? Numerous studies in animals have failed to reveal any psychological deficit as the result of interruption of corticocortical pathways. The recent observations by Sperry, Miner, and Myers[93] on the lack of effect of multiple subpial crosscutting of the striate cortex in the cat further emphasize our lack of knowledge of the function of the corticocortical pathways of the type that may be involved in the suprasylvian-striate reaction that has been shown by Purpura[37] to be inhibited by LSD. In view of this lack of knowledge of the role of corticocortical pathways in psychological processes it would be difficult to do more than speculate as to the relationship between inhibition of the corticocortical responses and the psychological effects of LSD.

Another approach to the elucidation of the relationship between the electrophysiological and perceptual effects of LSD might take the form of seeking to predict a drug's psychological effects in man on the basis of its effects on the electrical activity in the brains of animals. In this case, the drug's electrophysiological effects would be expected to be correlated with its psychological effects, but not to cause them. One may again take the depression of transcallosal response as an example of the electrical event and attempt to predict the psychological effect of a given drug in man on the basis of the drug's action on the transcallosal response. If inhibition of the transcallosal response were a property sufficient to predict psychotomimetic activity, then amphetamine, curare,[94] serotonin, and bufotenin, which Marrazzi has shown to depress the transcallosal response, should be psychotomimetic agents. The fact that only 2 of these 4 drugs are psychotomimetic agents indicates that inhibition of the transcallosal response is not a sufficient condition to allow prediction of psychotomimetic properties for a given drug in man. LSD has been shown to cause "alerting" or "arousal" responses in the EEG, but many agents that are not psychotomimetic have similar effects. Reserpine,[95] which is psychotherapeutic rather than psychotomimetic, causes desynchronization of spontaneous electrical activity, while *N*-allylnormorphine, a psychotomimetic, causes the appearance of a sleep pattern in the EEG.[72] The effects of drugs on lateral geniculate synaptic transmission are also of limited value in predicting the psychological effects of a given drug in man. Bufotenin[39] was found to be approximately one tenth as active as LSD in inhibiting geniculate transmission. On the basis of this ratio of activities, one would predict that doses of bufotenin of the order of 1 mg. should have psychotomimetic effects in man. Fabing[29] and Isbell,[30] however, have observed psychotomimetic effects in man only following much larger doses of bufotenin, of the order of 10 to 16 mg. The facilitatory effects of LSD on evoked cortical responses, as shown by Purpura,[37] are equally difficult to relate to the disturbances of visual perception that occur following the administration of LSD to man. Purpura points out this difficulty, stating that "whether or not the dual excitatory and inhibitory action of LSD demonstrated on the evoked cortical potentials of the cat's brain adequately explains the psychic disorganization and hallucinations described in man under LSD intoxication certainly can not be evaluated at this time."

If one did not start from the demonstrated electrophysiological effects of LSD, but attempted to predict, a priori, the electrophysiological effects that hallucinogenic drugs *should* have, the problem would be equally difficult. Visual hallucinations may be the end result of a wide variety of anatomical, psychological, and chemical changes in the nervous system. Klüver[96] has discussed some of the psychological approaches to an understanding of the processes underlying hallucinations, and he has indicated the diverse possibilities that reasonably may be set forth to explain hallucinations. Phantom limb pain, which may be considered as 1 type of hallucination, may involve any of a number of ascending neural elements, from the primary receiving neuron up to the cortical projection and association areas.[97] The diversity of origin of visual hallucinations is indicated by the fact that they may arise because of local disease of the eye[98] or may result equally well from the disturbances of the cortical function associated with migraine.[99] Neurological studies indicate that visual hallucinations may be caused by lesions placed at any point along the ascending visual pathway[100] and, in many cases, they may result from lesions that do not appear to impinge on the visual pathway. Thus, a priori, it would be difficult to single out any one aspect of the activity of the brain that needs to be disturbed in association with hallucinatory processes.

The preceding speculations have been limited to the possible electrophysiological basis of the hallucinogenic effects of psychotomimetic agents. The complexity of attempting to deal with the possible physiological correlates of the many other aspects of the psychosis induced by LSD precludes their discussion in this review. The difficulty involved in seeking an understanding of the neuropsychological basis of emotional disturbance is presented by Hebb,[101] who indicates some of the possible processes that might underlie emotional disturbance: "Emotion may be a disruption of cortical organization, which could occur in several ways: the occurrence of incompatible phase sequences; the absence of a sensory facilitation that has always contributed to the phase sequence; 'pain' stimulation that can be supposed to be innately disruptive of cortical activity; and chemical change of the blood content, altering the rate of firing of individual neurons and so disrupting a cortical organization that is fundamentally a matter of timing." In view of the difficulty involved in formulating the cerebral events that might underly emotional disturbance, it would seem even more difficult to assign the emotional disturbances that result from psychotomimetic drugs to a demonstrable disturbance of the electrical activity of the cerebrum.

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THE EFFECTS OF CERTAIN DRUGS ON CEREBRAL SYNAPSES

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As Edward Evarts has so clearly indicated in his contribution to this volume, we are all interested in determining the neurophysiological correlates of mental disturbance in the hope of thereby gaining an inkling of its underlying mechanisms and developing a rational therapy for it. Humphry Osmond has drawn a dramatic picture of the opportunity presented by the situation made possible by the psychotomimetic drugs, which afford us the means of inducing at will a reversible model psychosis. This model psychosis, even though it bears only a fragmentary resemblance to schizophrenia, nevertheless simulates certain aspects of mental disturbance by perhaps similar mechanisms. Furthermore, the so-called model psychosis also can be shortened and terminated at will by the tranquilizers for which clinical effectiveness in schizophrenia is claimed. The use of drugs as tools thus creates favorable conditions for studies of mental illness.

Our efforts, as investigators, are directed more toward an intelligent application of the hypotheses of mechanism rather than toward simple clinical evaluation. The conditions that we wish to interpret are fully and truly exhibited in man but, before we can take full advantage of controlled conditions induced in humans, it is necessary to perform some prototype experiments in animals since, in such experiments, more procedures are permissible and in them those experiments intended for man can be constructed and rehearsed. This purpose, the needed groundwork before work with humans can be done, is my justification for presenting some data on animals and making comparisons with clinical conditions and experimentally induced conditions in man.

FIGURE 1 summarizes the data that led my co-workers and me to a hypothesis that served as the point of departure for studies in this field.[1] It shows that in our survey of a variety of sites in the nervous system we find, as far as we have gone, that a consistent reciprocal relationship exists between excitation or enhancement by acetylcholine and acetylcholinelike substances, including anticholinesterases, on synaptic-transmission phenomena and inhibition by epinephrine, norepinephrine, all sympathomimetic amines in varying degrees, and related substances.[2] It seemed plausible that any perversion of metabolism that would distort the balance of endogenous chemical or neurohumoral control of synaptic-transmission processes could lead to abnormal cerebral performance or mental disturbance, and that chemicals or drugs could alter the equilibrium of transmission and thereby alter cerebral and mental function in the direction of health or disease.

The limitations of communication with animals make it exceedingly difficult, though not impossible, to relate the behavioral disturbances that can be produced in them with mental disturbance in man. Since our basic premise, however, is that all cerebral function, including both behavior and mental processes, is made up of functional units accumulated in series and in parallel

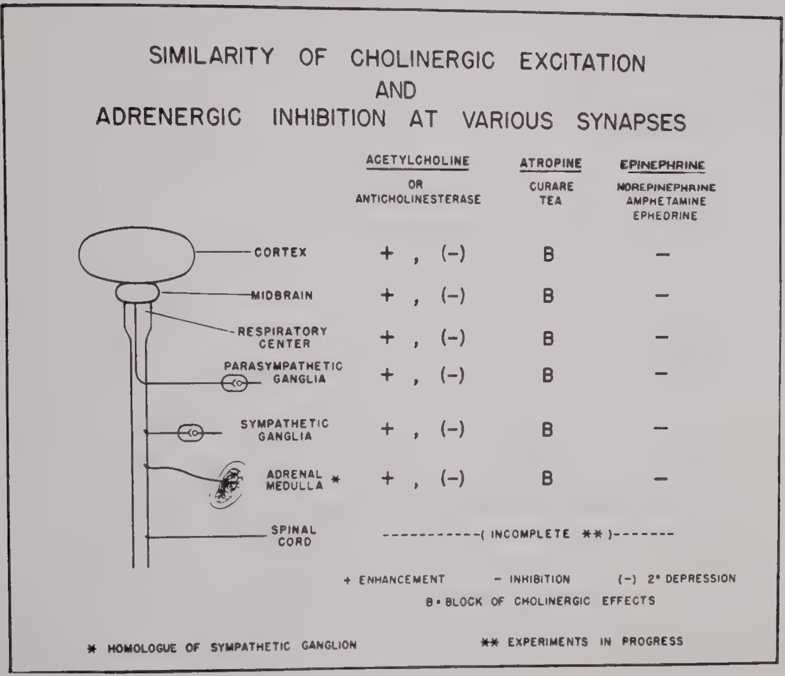
SIMILARITY OF CHOLINERGIC EXCITATION AND ADRENERGIC INHIBITION AT VARIOUS SYNAPSES
ACETYLCHOLINE OR ANTICHOLINESTERASE
ATROPINE CURARE TEA
EPINEPHRINE NOREPINEPHRINE AMPHETAMINE EPHEDRINE
CORTEX + , (−) B −
MIDBRAIN + , (−) B −
RESPIRATORY CENTER + , (−) B −
PARASYMPATHETIC GANGLIA + , (−) B −
SYMPATHETIC GANGLIA + , (−) B −
ADRENAL MEDULLA * + , (−) B −
SPINAL CORD -----------(INCOMPLETE **)-------
+ ENHANCEMENT
− INHIBITION
(−) 2° DEPRESSION
B = BLOCK OF CHOLINERGIC EFFECTS
* HOMOLOGUE OF SYMPATHETIC GANGLION
** EXPERIMENTS IN PROGRESS

FIGURE 1

combinations to form patterns, I believe it of value to study such units, that is, the synapses.

The transparent model of the brain of the cat (FIGURE 2) illustrates a relatively simple synaptic* preparation that we have found convenient for study. I must emphasize, at the outset, that we consider the experiment pertinent to the extent that it deals with visual pathways, since the powerful psychotomimetic drugs exhibit an important visual component in the hallucinations, dramatically so with mescaline. More important than that, our findings impress us with the similarities rather than the differences between synaptic performance and susceptibility to chemicals, either endogenous or exogenous (drugs). Therefore, we are really using the transcallosally activated cerebral synapses in the visual area of the cat merely as representative of cerebral synapses in general, all of these synapses having qualitative similarities and varying principally by differences of threshold. We do not intend to suggest that an alteration in this specific pathway is necessarily responsible for mental disturbance.† A little later I shall outline a general working hypothesis based

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* "Synapse" is used throughout in the sense of designating the total complex involved at the functional articulation of 2 neurons, that is, presynaptic nerve ends, transmission process, postsynaptic dendrites, and soma.

† Chronic interruption in a system such as the transcallosal, as mentioned by Edward Evarts, should not necessarily be expected to produce the same changes as an acute interruption by drugs unaccompanied by surgical trauma and subsequent degenerative processes.

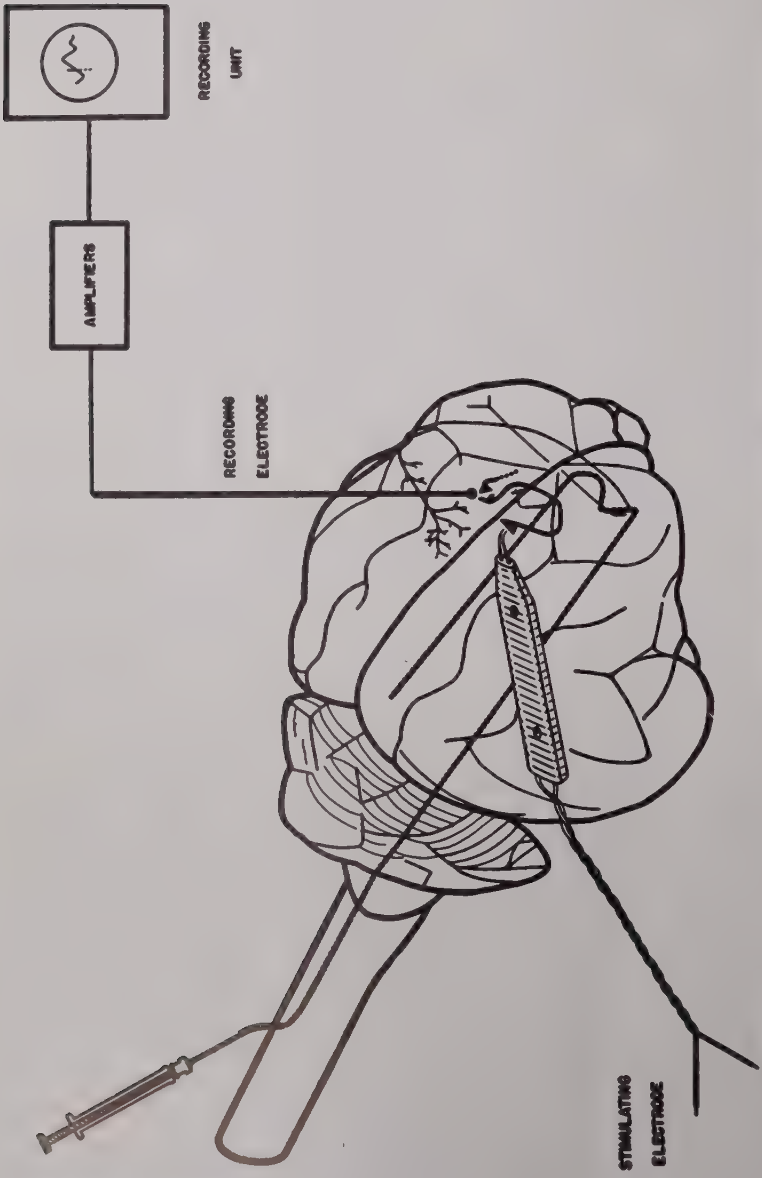


FIGURE 2. Transparent model of the brain, showing 2-neuron intercortical (transcallosal) pathway. The schematic arterial blood supply is the pathway for the injected material.

on a disruption of normal patterns that results from alteration in amounts of synaptic regulators or in the thresholds of the neurons upon which they act.

Since Edward Evarts has already outlined our technique I can be very brief in pointing out certain features. Because the brain is a communication system it seems most appropriate to measure function by recording the handling of a test message. The test message is supplied in the form of a submaximal electrical stimulus applied to 1 optic cortex in a cat that has received a light dosage of pentobarbital sodium. This stimulus initiates a conducted response in the association or transcallosal tract that connects the stimulated point to a symmetrical point in the contralateral cortex where, after synaptic transmission, the stimulation evokes a cortical potential, as first described by Curtis and Bard.[3] To help distinguish between peripheral effects that would contribute to the afferent drive constituting the background against which the impulses are elicited and the strictly central effects, we take advantage of the fact that an intracarotid injection will achieve a transient, higher concentration of drug on the ipsilateral or recording side but, when diluted by the blood in the general circulation, the concentration of the drug is brought down to levels that are below the threshold for the peripheral effects. Under the conditions of our experiment, the amounts of the drug passing through the circle of Willis to the other cortex are unimportant.

In this way it becomes possible to demonstrate (FIGURE 3) that epinephrine, a chemical natural to the body, one known to produce anxiety when accumulated in sufficient amounts, either endogenously or exogenously, also produces cerebral synaptic inhibition, as indicated by the reduction in the signal (surface negative wave) corresponding to outflow, while the inflow (surface positive wave) is essentially unaltered. The same type of synaptic inhibition is shown for another cerebral neurohumor, norepinephrine, in the next line of the same

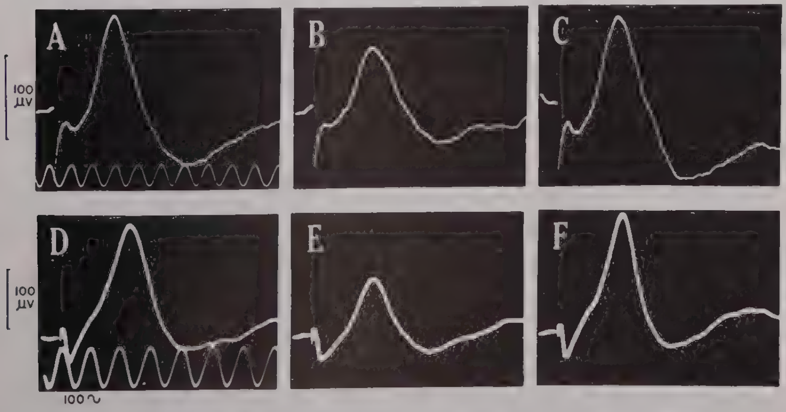
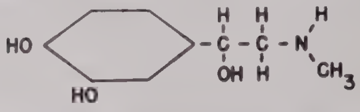
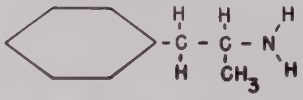


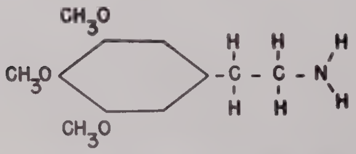
FIGURE 3. The cerebral synaptic action of epinephrine and norepinephrine in a 2-neuron intercortical (transcallosal) system. Potentials are evoked in the optic cortex by the electrical stimulation of a symmetrical point in the contralateral cortex. Epinephrine (10 μg./kg.) was injected into the ipsilateral carotid artery after *A*, and norepinephrine (150 μg./kg.) was injected after *D*. *A* and *D* are controls, *B* and *E* represent inhibition, and *C* and *F* show recovery.



EPINEPHRINE (dihydroxy-phenyl-ethanol methyl amine)



AMPHETAMINE (phenyl-isopropyl amine)



MESCALINE (trimethoxy-phenyl-ethyl amine)

FIGURE 4. Types of phenyl-ethyl amines producing mental effects.

figure, but this action is evidently weaker than the other, requiring a larger dose to produce approximately the same degree of inhibition.

In FIGURES 4 and 5 are shown some structural chemical similarities of compounds with which other contributors to this volume have already dealt. Attention is called to the close structural similarity (FIGURE 4) of epinephrine to amphetamine, which is also capable of producing anxiety, and mescaline, which does so regularly and with dramatic intensity, producing a full-blown "model psychosis." These drugs in turn are related to the group shown in FIGURE 5, in which epinephrine is once more presented alongside a first-oxidation product, adrenochrome, which is an indole. Below these are pictured *d*-lysergic acid diethylamide (LSD-25), the very highly potent psychotogen, which can be considered to be built on an indole nucleus, and 5-hydroxytryptamine, or serotonin. The epinephrinelike psychotogens thus can be chemically related to the indolelike ones, including established drugs such as LSD-25, reputed drugs such as adrenochrome, described at the beginning of this monograph by Humphry Osmond, and by myself elsewhere,[2] and the naturally occurring indole found in the brain,[4a, b] which is postulated by Woolley and Shaw[5] to be sufficiently related to LSD-25 possibly either to compete with or to add to its action. We now looked to see whether there was any functional parallelism or neurophysiological correlate of this relationship by using the objective test of cerebral performance afforded by the evoked-potential technique in the cat. FIGURE 6 presents the data showing that there is an actual correspondence in structure, and that all the compounds produce synaptic

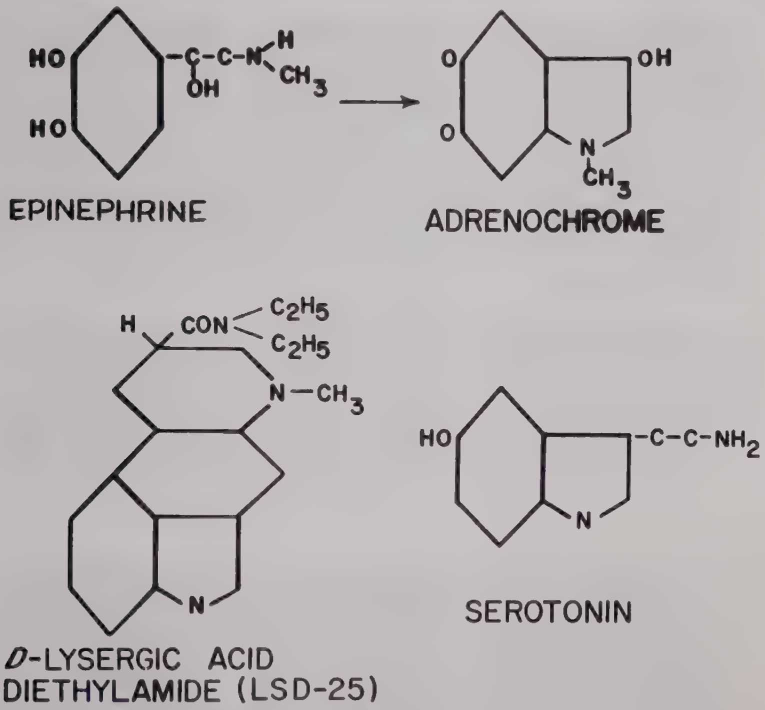


FIGURE 5.

inhibition identical in kind to that produced by epinephrine, but vary in degree of effectiveness, so that for the approximately equivalent effects shown it required milligram amounts of mescaline, but only microgram quantities of LSD-25, which duplicates the relative potency of these compounds as found in clinical experience. The dosages used throughout our experiments are intentionally of a size selected to produce incomplete actions, so that recovery back to the control level can be secured more readily.

Very interesting is the finding with serotonin, which turns out to be the most effective cerebral synaptic inhibitor of all, being effective in as little as 1-μg. doses. Accordingly, rather than being an antagonist, this indole, or something like it, may represent the type of endogenous substance that is instrumental in bringing about some forms of spontaneously occurring mental disturbance.

Furthermore, since serotonin is naturally present in the brain[4a, b] and is so highly potent (about 20 to 25 times as potent as epinephrine in the same experiment), serotonin becomes, as we pointed out over a year ago,[2] an even better candidate than either epinephrine or norepinephrine, which are also found in the brain,[4a, b, 6] for the role of inhibitory neurohumor.* This finding would

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* Serotonin must penetrate the blood-brain barrier at least in the small amounts required to exercise the cerebral action described.

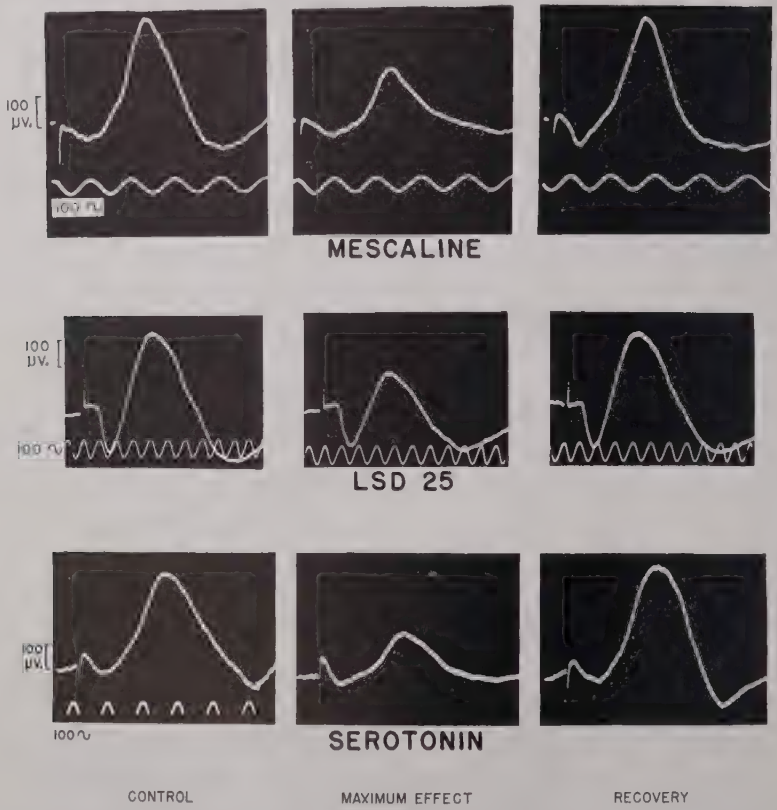

Figure 6.

point even more closely to a derangement of neurohumoral balance at synapses as a potential mechanism of cerebral or mental derangement.

Unfortunately, except for the intraventricular injections described by Sherwood,[7] there have been, thus far, no documented reports of serotonin-induced mental disturbance* in man that are clearly separable from the natural anxiety initiated by the profound peripheral effects such as circulatory disturbance, other autonomic effects, and emesis. There are, however, such reports for a close analogue of serotonin, dimethyl-serotonin, or bufotenin, which is used for its mental effects by some primitive peoples and has been observed by Fabing[8] to produce such disturbances in man experimentally. These 2 substances, as well as adrenochrome, a presumed metabolite of epinephrine that

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* The fact that patients with carcinoid have large amounts of circulating serotonin without showing marked symptoms of mental derangement could represent an adaptation to very high levels of serotonin that has developed and accumulated gradually. This suggestion would account for the relative immunity of such patients to the possible central effects of high doses of serotonin injected intravenously.

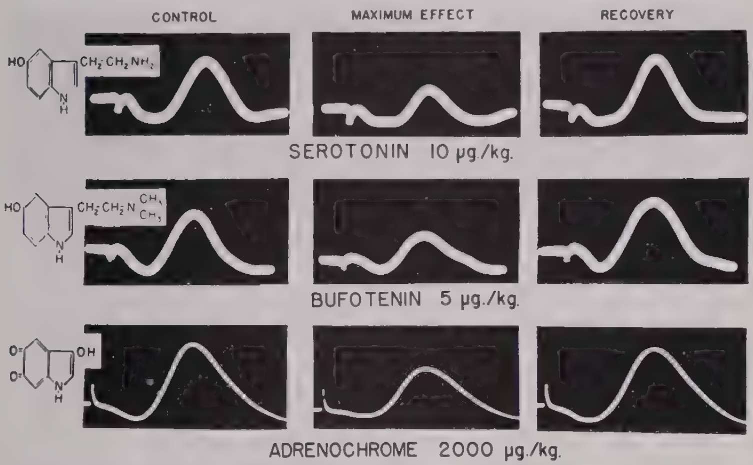
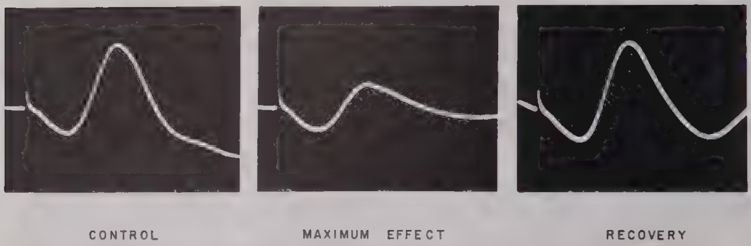


FIGURE 7. Cerebral synaptic inhibition by indoles in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were given in the ipsilateral common carotid artery.

Hoffer, Osmond, and Smythies[9] report as reproducing some aspects of the clinical syndrome of schizophrenia when injected intravenously in man, are compared in the cat in FIGURE 7. Again, all these compounds have the identical qualitative effect, namely, synaptic inhibition, but bufotenin, tested in the same animal, exhibits twice as much effectiveness as does serotonin, which required 10 µg. for its effect on this occasion. Adrenochrome, though it does induce synaptic inhibition, requires so large a dose, 2 mg., that it seems an unlikely candidate for the role of endogenous psychotogen responsible for a form of mental illness, although a substance somewhat like it might be responsible.

The great effectiveness of serotonin not only suggests that this is the type of chemical structure implicated, with the reservations already noted, but that it constitutes 1 link, another being its natural occurrence in the brain, in the chain of evidence identifying it as a cerebral neurohumor. A required piece of information to round out this evidence would be the measurement of the actual liberation of serotonin during, or prior to, the recorded synaptic activity. This is a tedious and difficult type of experiment, and it is attended by special handicaps in work on the brain. Another approach leading to a similar conclusion, however, is quite readily followed. This approach is the accumulation of what must be naturally occurring serotonin, strategically located at the synapses, by the poisoning of the enzymes that normally lead to the destruction of serotonin and account for the ready reversibility and short duration of the action of serotonin. This is the technique that has been used so successfully in the study of the function of acetylcholine in the brain, and it is in this manner, by the use of a powerful anticholinesterase, that we demonstrated the presence and operation of acetylcholine at cerebral synapses.[1] Serotonin is known to be very susceptible to destruction by monoamine oxidase, which is abundantly



CONTROL MAXIMUM EFFECT RECOVERY

FIGURE 8. The cerebral synaptic action of iproniazid in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the optic cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The iproniazid (5 mg./kg.) was injected into the ipsilateral carotid artery.

present in the brain.[10] We therefore attempted to inhibit this enzyme by iproniazid (Marsilid). FIGURE 8 shows the result of a preliminary experiment in which we injected iproniazid into the common carotid artery of the cat in the same way that we had done previously with serotonin. The effect produced duplicated the serotonin effect as if, indeed, the serotonin at the synapse had been preserved by the inhibition of monoamine oxidase by the iproniazid. I believe this finding offers another piece of important evidence that serotonin is present naturally, not only in the brain, but at strategic sites where it is capable of influencing synaptic transmission. We have not as yet measured, as we need to do, how much this dose of iproniazid, given in this way, inhibits cerebral monoamine oxidase in the cat.

We believe that the somewhat discouraging attitude of some investigators toward basing clinical prediction on animal experimentation is not entirely justified, since this procedure is a natural result of the comparison of objective criteria such as we have just described with clinical evaluation based upon questionnaires and much undoubtedly shrewd clinical observation, both of these types of data being very difficult, indeed impossible, to quantitate. Accordingly, we are more impressed by the degree of correspondence obtainable rather than by the discrepancies that are to be found. Thus our evoked-potential experiments in the cat rank the psychotogens and psychotomimetic substances studied so far, in general, in the order of clinical effectiveness, and they suggest that at least part of the mechanism responsible for mental disturbance is to be found in an imbalance in the regulation of synaptic transmission. One such imbalance we have already described.

If this hypothesis is truly useful, and if the animal preparation used bears other than a merely empirical relation to the clinical data, we should expect that the various tranquilizers for which varying degrees of clinical success have been claimed would have some action here also. We proceeded to test this extension of our thinking, and we found that all of the several types of tranquilizers are capable, when administered prophylactically to cats, of partially preventing, in the doses used, the cerebral synaptic inhibition of a test dose of mescaline.

FIGURE 9 shows this reaction, using chlorpromazine (Thorazine). The figures now read from top to bottom instead of from left to right, as in the previous

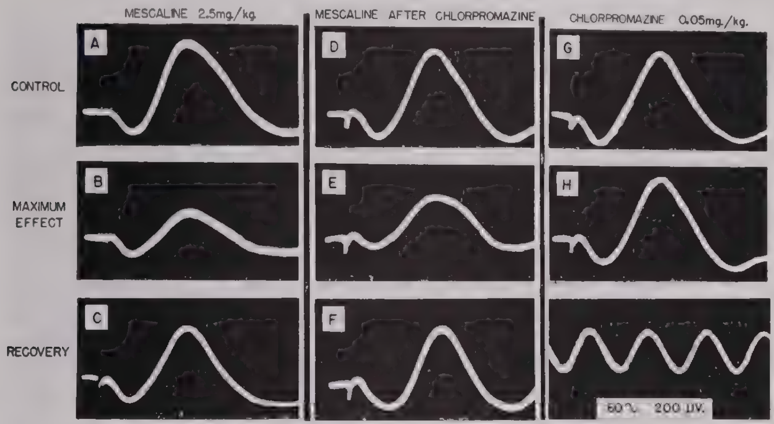


FIGURE 9. The prevention of the mescaline effect by chlorpromazine in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were made in the ipsilateral common carotid artery.

figures. The first column shows the control, the mescaline inhibition at *B*, and the recovery at *C*. After this, chlorpromazine is given in doses which, per se, have no apparent effect on synaptic transmission, as shown by the new control *D* in the second column, but now when mescaline is given again, the synaptic inhibition *E* is much reduced when compared to *B*. Without the tranquilizers, the same degree of inhibition of mescaline can be repeated several times in succession, provided that complete recovery is allowed between injections. Records *G* and *H* show again that this dose of chlorpromazine did not impede synaptic transmission despite the ability of the drug to protect against mescaline. If the dose is increased twentyfold it does have a depressant

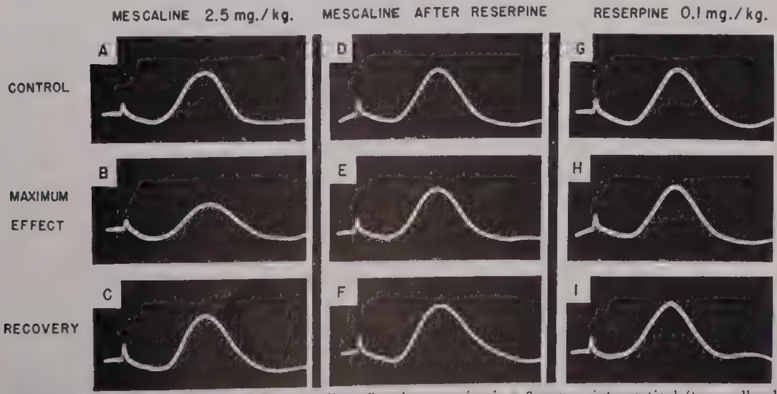


FIGURE 10. The prevention of the mescaline effect by reserpine in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were given in the ipsilateral common carotid artery.

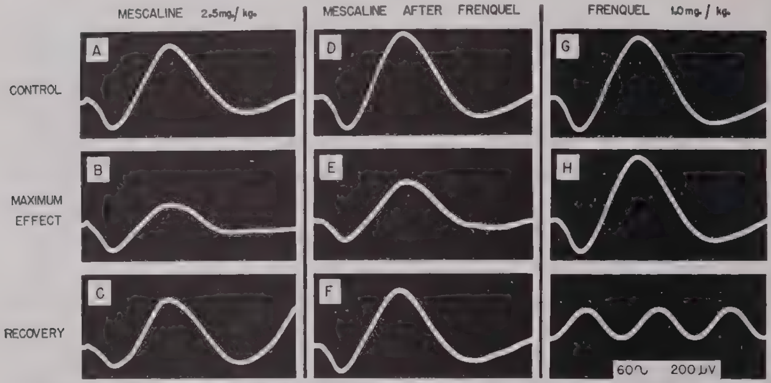


FIGURE 11. The prevention of the mescaline effect by Frenquel in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by the electrical stimulation of the contralateral cortex every 2 seconds. The injections were given in the ipsilateral common carotid artery.

action on synaptic transmission. The same prophylactic action is obtained with reserpine (Serpasil), as shown in FIGURE 10, and with azacyclonol (Frenquel), as shown in FIGURE 11. Another point of correspondence with clinical findings is that the margin of safety, in this case the range between the prophylactic and the synaptic-depressant dose, is large, the depressant dose being 15 to 20 times the prophylactic dose with both chlorpromazine and azacyclonol, but the factor is only 2 with reserpine. The latter drug approximates the action of the barbiturates, which can reduce the degree of demonstrable inhibition from mescaline by reducing synaptic transmission in the first place.

I feel justified in saying, then, that the preparation described is pertinent to the clinical situation in that it ranks the psychotomimetic substances in the order of their clinical effectiveness, and that the action of mescaline, the only drug that we have tried so far, is prevented by the tranquilizers.

By use of the evoked-potential technique, we have demonstrated that:

(1) There exists an equilibrium of neurohumoral control of transmission at cerebral synapses and throughout the nervous system, as far as I have surveyed it, that is susceptible to distortion and imbalance by disturbance in the amounts of chemical regulator or the susceptibility of neurons.

(2) The psychotogens and psychotomimetic substances discussed, structurally and functionally resemble the actions of the fairly well-established inhibitory synaptic neurohumors, epinephrine and norepinephrine, and of serotonin, the new one that we have described.

(3) Serotonin or its dimethyl derivative, bufotenin, comes close, even closer than does LSD-25, to representing the type of endogenous psychotogen that might be a natural cause of some forms of mental disturbance.

We speculate that such disturbance can be produced by direct perversion of normal patterns of neuronal activity by the undue influence of synaptic inhibitors or, indirectly, by such inhibitors impeding the flow of impulses from higher controlling centers and releasing the more primitive, simpler, and less well-adapted patterns of activity that we call abnormal.

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THE EFFECT OF THE HALLUCINOGENIC DRUGS LSD-25 AND MESCALINE ON THE ELECTRORETINOGRAM

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and

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There is a controversy in the literature concerning the precise manner in which *d*-lysergic acid-*dl*-hydroxybutylamide-2 (LSD-25) and mescaline induce visual hallucinations in normal human subjects. It is essential that this problem be clarified since the designation of these 2 drugs as "psychotomimetic" is influenced by the concept that the central nervous system participates in the production of these hallucinations.

Our present knowledge of this problem has arisen from clinical and experimental studies on humans and on cats. Klüver[5] has described in great detail the visual hallucinations induced in human subjects. These hallucinations take 2 forms: early appearing geometric patterns in many colors, and later appearing panoramas. A publication by Stoll[10] depicts hallucinations produced by LSD-25 with line drawings of honeycombs, lattices, filigrees, and ruffles. These patterns appear to the subject in luminescent yellow, green, and red colors. These colors are similar to the bright phosphores that appear in the visual field after the mechanical stimulation of the eyeball. These hallucinations do not appear to subjects unless their optic nerves are intact.[1, 2] Physiologists have studied the effect of LSD-25, in both nontoxic and toxic doses, on the visual pathways of cats. Purpura[6, 7] has deduced that this drug facilitates synaptic transmission in the central nervous system, and Rovetta[8] finds spikes on the occipital cortex that are similar to the spikes caused by strychnine.

A perusal of these clinical and experimental data indicates that the source of the visual hallucinations might be in the retina itself rather than in the central nervous system. The present study investigates the extent to which the retina is involved in the production of visual hallucinations induced by LSD-25. The study is based on the principle that an electrical potential accompanies activity in any nervous element.

Action potentials may be detected anywhere on the surface of the eyeball following marked changes in the illumination of the retina.[4] These potentials indicate activity in the retina, and they occur only when retinal elements are transmitting electrical impulses. It follows, therefore, that such an electroretinogram would be induced by the hallucinogens when the illumination of the eye remained constant only if the retina were participating in the production of spontaneous visual experiences. It seemed worthwhile, therefore, to investigate the effects of hallucinogenic drugs on the retinas of cats.

Methods

(1) In order to ascertain a dosage of LSD-25 that would be nontoxic for cats, and to determine whether cats experience visual hallucinations with it, 0.10 mg. of LSD-25 was injected into the peritoneal cavity of each of 3 cats. The behavior of these animals was observed for 2 hours.

(2) Seventeen cats were anesthetized with pentobarbital sodium, injected intraperitoneally (42 mg./kg.). Two nonpolarizable silver-silver chloride electrodes were sutured to the sclera of each cat, and they served to pick up the electrical potentials from the retina. A Grass electroencephalograph recorded these potentials. The efficacy of this procedure in demonstrating the activity of the retina was tested by noting the potentials recorded following the photic stimulation of the retina.

The effects of LSD-25, mescaline, pentobarbital sodium, and dihydrocannabinol on the retinogram and on the resting potentials of the eye were compared in an effort to determine whether the hallucinogenic drugs act directly on the retina.

(3) In 9 cats, 2 electrodes were wound around the optic nerve of 1 eye. In these cases all muscle and nerve connections of that eye were severed to eliminate any contribution that nonvisual elements might make to nerve-action potentials. The other eye of each of these cats had electrodes applied as in Method 2. LSD-25 was injected, and its effect on potentials in the optic nerve was studied.

(4) In 7 cats, 2 electrodes were placed on each eye, as in Method 2. Simultaneously a bipolar electrode was placed for pickup from an exposed area on the occipital visual cortex. The effect of LSD on potentials in this area was noted, and then the effect of the section of the optic nerves on the induced effect of LSD-25 was recorded.

Results

(1) The 3 cats to whom LSD-25 was administered all acted in the same way. Two minutes after the intraperitoneal injection they began to act somewhat excitedly. Their pupils and lid slits became widely dilated. After 8 minutes they rubbed their eyes frequently and vigorously. They peered into their cages, acted as though they were startled by objects in their visual fields, and grasped at the air in front of them with their paws. To an observer of normal behavior in cats, it was evident that these animals were experiencing evanescent visual hallucinations. All 3 cats recovered uneventfully from this dose of LSD-25 in about 2 hours.

(2) Pentobarbital sodium, which was used on 17 of the cats, alone of the drugs used induced no spontaneous electrical potentials in the retina of the cat. Photic stimulation of the eyes of the cats induced a normal retinogram. The recordings in this case are, therefore, identical with the findings in a normal, undrugged human subject whose eyes are exposed to unchanging dim illumination.

Mescaline, which was used on 6 cats in a dose of 150 mg./kg., was injected intraperitoneally 50 minutes after the anesthetizing dose of pentobarbital

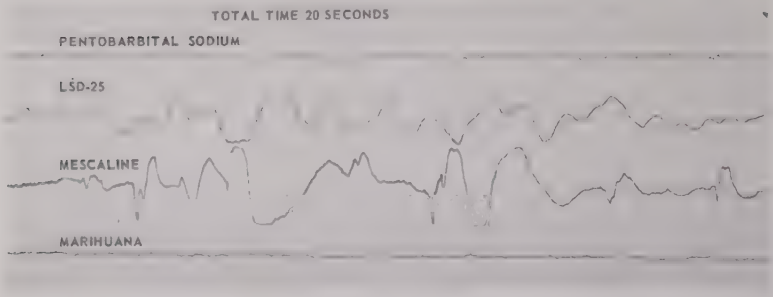

FIGURE 1. Spontaneous potentials from 4 cats, each of which was under the influence of a different drug.*

sodium. From 2 to 10 minutes later spontaneous action potentials appeared on the recording. The potentials occurred at a rate close to 1 per second (FIGURE 1), and had the characteristics of the *B*-wave of the electroretinogram; that is, a sharp positive potential followed by a slower negative one. The spontaneous potentials proceeded in spurts of 20 seconds each for from 4 to 6 minutes, and then the normal resting potentials reappeared. At this time the response induced by photic stimulation of the retina consisted of a retinogram of normal form, but of increased voltage, followed by a varying number of the *B*-like waves (FIGURE 2). In 2 of the cats the pickup was taken simultaneously from the optic nerve and from the sclera. In this case the spontaneous potentials of identical form appeared first at the sclera and, a few milliseconds later, in the optic nerve.

LSD-25 (0.10 mg. in each of 7 cats) was injected intraperitoneally. It induced precisely the same effect on the retinal potentials as did mescaline.

Dihydrocannabinol (marihuana) was used in 1 cat. The recording following injection of this drug was identical with the recording made following the injection of pentobarbital sodium. Neither of these drugs produced the geometrically patterned visual hallucinations that mescaline and LSD-25 created.

In human subjects the onset of the bright-lights sensations was accompanied by spontaneous retinal potentials as well as by a slight decrease in the voltage of the alpha rhythm from the occipital area as described by other investigators.[3, 9, 11]

(3) Spontaneous action potentials appear in both the optic nerve and the retina (FIGURE 3). In numerous instances the same wave form was picked up first from the retina and then from the optic nerve. These results indicate that the spontaneous potentials do indeed originate in the retina itself and not from any extraocular muscle or pupillary activity.

(4) Large spikes appear on the visual cortex of the cats after a dose of LSD (FIGURE 4). These have been reported by Rovetta[8] for mescaline. These spikes are higher and sharper than the retinal potentials, and they disappear when the optic nerves are severed. It is apparent, therefore, that the spon-

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* The figures illustrating this article appeared in the *American Journal of Ophthalmology* Part, II, volume 22, article 5, pp. 206 to 211, and are reprinted by special permission.

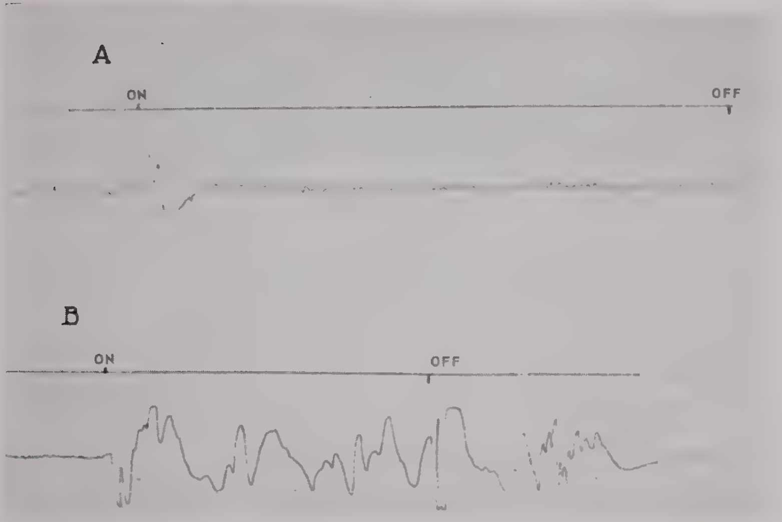

Figure 2. The potentials induced by turning a light on and off the retina of the cat, *A*, while the animal was anesthetized with pentobarbital sodium, and *B*, 15 minutes after the administration of 0.1 mg. of LSD-25.

taneous visual-system activity initiated by LSD-25 starts in the retina. The activity travels through the visual pathways with facilitation at each synapse.[7] This results in higher, sharper spikes at each nucleus with maximal activity in the cortex. The activity in the cortex cannot appear unless the connection with the retina is intact.

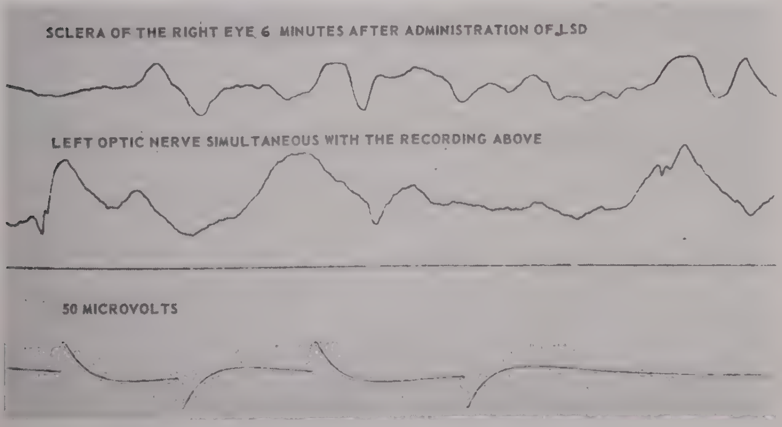

Figure 3. Simultaneous pickup from the optic nerve and the retina, showing the spontaneous potentials.

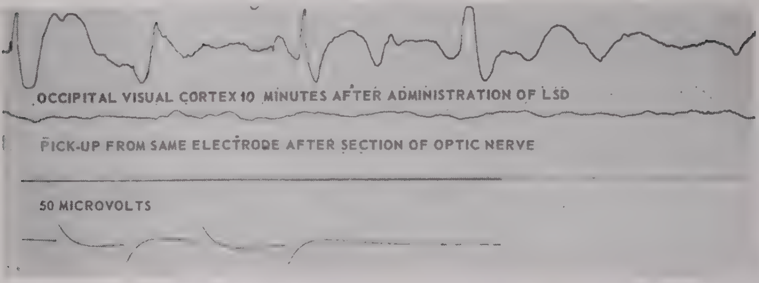

FIGURE 4. Pickup from the occipital visual cortex 10 minutes after the administration of LSD-25 before and after section of the optic nerve. The upper record shows the spikes induced by the LSD-25, and the lower record shows the absence of spikes after the section of both optic nerves.

Conclusions

These experiments demonstrate that cats are capable of having hallucinations as the result of a nontoxic dose of LSD-25. This same dose of LSD-25 initiates spontaneous action potentials in the retinas of anesthetized cats. The potentials are certainly not induced by muscular activity. Moreover, the cortical activity induced by the drug is dependent upon nervous transmission from the retina, and it does not appear when the connection with the retina is severed.

These findings are consistent with the clinical data. The objective description of geometric hallucinations induced by LSD-25 in reliable human subjects is best explained on the basis of intraocular structures. In humans with section of the optic nerve LSD does not induce hallucinations. These facts, coupled with the experimental data, demonstrate that LSD-25 does not induce hallucinations solely by virtue of an effect on the central nervous system. The facilitation of central synapses produces no effect on the visual pathways unless a visual experience starts in the retina. This facilitation that we have demonstrated in the retina and that Purpura[7] reports in the geniculate body and cortex accounts for the complex hallucinations that sometimes appear. We may assume that such hallucinations depend upon an intact connection from the eye to the central nervous system.

These findings lead us to conclude that the hallucinations induced by LSD-25 are not similar to illusions induced by psychosis, since the drug has a marked peripheral action.

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Discussion of the Paper

Mary A. B. Brazier (*Massachusetts General Hospital, Boston, Mass.*): Electroretinograms are potentials of long duration, and their form is severely distorted by resistance-capacitance coupled amplifiers with the time constants shown in the figures illustrating this article. In addition, there will be a loss of amplitude of over 20 per cent. More important, perhaps, is the fact that these time constants will cause a phase displacement leading by at least 50° in the case of the slow electroretinogram and a phase lag for the faster potentials of the nerve. This fact should be borne in mind in drawing any conclusions as to the time relations of the electroretinogram to the potentials of the optic nerve when recorded in this way. It would be interesting to review these findings of potential changes in the electroretinogram following the administration of LSD-25 and mescaline in the light of Granit's demonstration of the centrifugal efferents to the retina.

Reference

Granit, R. 1955. Centrifugal effects on the retina. J. Neurophysiol. **18:** 385–411.

Walter S. Boernstein (*Research Center for Mental Health, New York University, New York, N. Y.*): In my own work in this area I have been able to show that optical hallucinations can be induced in subjects adapted to darkness by nonoptic sensory stimuli of a certain type—that is, "bright" stimuli of *any modality*—as well as by those *movements* that increase the tonus of the muscles, such as bending an arm against resistance.

"Dark" sensory stimuli either have no effect at all, or they induce optical hallucinations of a different type that usually is more fleeting.

In one case of cortical hemianopia caused by a shell fragment, optical hallucinations could be induced in the blind field.

Further experiments showed that the migration of *pigment*, and possibly the contraction of the cones in the retina of the frog, and a corresponding migration of pigment in the melanophores of the skin of the toad (*Xenopus laevis*) could be induced by the same type of chemical and physical stimuli that induces optical hallucinations in man.

Consequently, a close relationship seems to exist between the processes in the retinas of vertebrates during vision, the nervous mechanism that regulates the general body tonus, and those cells in which the receptor and motor functions are not yet separated; that is, the melanophores.

These experiments and the observation of the case of hemianopia, together with other physiological and psychological experiments, seem to suggest strongly that the retina is not the only region of the organism that is responsible for the occurrence of optical hallucinations.

Reference

BOERNSTEIN, WALTER S. 1936. On the functional relations of the sense organs to one another and to the organism as a whole. J. Gen. Psychol. **15: 117–131.**

JULIA T. APTER: Mary A. B. Brazier's comments are most welcome since they indicate that she accepts the potential changes illustrated in this article as evidence of a significantly altered electroretinogram. The facts that she points out are most pertinent to the data of this study. We agree that it is incorrect to assume from time relations alone that the optic-nerve potentials were initiated in the retina. We have avoided using that criterion in concluding that these drugs act on the retina. In fact, we have found that the amplitude of the spontaneous retinal potentials varies directly with the intensity of the steady illumination on the retina.[1] This finding, coupled with the observation that spontaneous optic-nerve potentials cease after the nerve has been cut at the globe has influenced our premise that the retina participates in the production of drug-induced hallucinations. Further hypotheses concerning the precise origin and character of these potentials should await more detailed studies including, as Brazier recommends, investigations of the role that fibers centrifugal to the retina may have in this phenomenon.

We are glad to agree with Walter S. Boernstein that the whole organism must contribute to the production of many types of induced hallucinations. Heinrich Klüver[2] observed that most sensory phenomena induced by drugs had the same quality as the visual sensations when they occurred together. The subjects reported that they felt the grillwork as well as saw it. All these observations indicate that the peripheral action of LSD and mescaline need further study.

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EXPERIMENTAL ANALYSIS OF THE INHIBITORY ACTION OF LYSERGIC ACID DIETHYLAMIDE ON CORTICAL DENDRITIC ACTIVITY*

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Introduction

In previous reports, evidence has been presented that demonstrates a differential sensitivity of dendritic activity to the inhibitory effects of lysergic acid diethylamide (LSD).[1] Whereas LSD in high concentrations (70 to 100 μg./kg.) depresses all evoked cortical potentials in unanesthetized cats that have been paralyzed with succinylcholine, at lower concentrations (2 to 50 μg./kg.), LSD facilitates the axosomatic synaptic activity characteristic of specific afferent synaptic systems, and it inhibits axodendritic activity in nonspecific, interareal and intercortical afferent systems. The hypothesis advanced, that is, that the action of the psychotomimetic agent LSD in low concentrations is dependent on the nature of the predominant synaptic interrelation present in any pathway, raises a number of important physiological and pharmacological questions concerning the mechanism of the inhibitory action of LSD on dendritic activity.

The reasons for these questions are as follows:

(1) It is generally asserted that the alteration in the spontaneous brain-wave activity in the experimental animal, produced by LSD, resembles in many respects the electrographic picture generally associated with behavioral arousal.[2, 3]

(2) It has been proposed that the spontaneous potential oscillations recorded from the surface of the brain result from the summated activity of the electrically excitable membrane of the apical dendrites.[4-6]

Since it has been shown that LSD exerts an inhibitory action on synaptically activated dendritic responses,[1] it is of importance to define the nature of the dendritic potential in order to eliminate the possibility of a direct effect of LSD on electrically excitable membrane believed to be the site of the 12 to 15 msec. surface-negative graded response.[4, 5, 7]

To accomplish this and to elucidate the mechanism of inhibition of LSD it is of further interest to present experimental data relating to the following: (1) the demonstration of inhibitory as well as excitatory synapses on dendrites; (2) the possible source of inhibitory afferents synapsing on dendrites; and (3) the neurohumoral agents concerned with axodendritic synaptic transmission.

The purpose of this report is to present new information and to summarize other recently obtained data on all of these subjects in an attempt to determine more precisely the mode of action of LSD on dendritic activity and the alterations produced by this agent on the electroencephalogram (EEG).

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† Supported by a special research fellowship granted by the National Institute for Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

METHODS

All experiments were performed on unanesthetized cats prepared in a manner similar to that previously reported.[1] Cross-perfusion experiments were performed on pairs of intact or *encéphale isolé* cats. Double femoral artery-to-vein cannulations were effected after deep heparinization. Preparation of these animals has been described more fully elsewhere.[8]

Stimulation of the pyramidal tract in the medulla was accomplished by insertion of a pair of fine Teflon-coated silver wires from the dorsum at the mid-olivary level. By means of a suitable switching arrangement, antidromic or orthodromic responses could be obtained by recording from or stimulating the motor cortex and recording from or stimulating the medullary pyramid. In all other instances, monopolar recording was utilized with reference to the bone over the frontal sinus. For single-shock and repetitive stimulation of the brainstem, a concentric electrode (0.5 mm.) was placed in appropriate sites by means of a 3-dimensional micromanipulator. All stimulating sites were checked either grossly or histologically. Unless otherwise stated, the multiple-sweep technique was utilized throughout in order to evaluate the range of variability of the evoked responses.

RESULTS

Nature of the Dendritic Potential

To determine the role that synaptic activity played in the genesis of the surface negative wave of 12 to 15 msec. duration, experiments were performed on unanesthetized and artificially ventilated cats that had been paralyzed with succinylcholine. Dendritic potentials were evoked by "direct" stimulation of the cortical surface, by antidromic stimulation of pyramidal neurons, or by subsurface stimulation. Utilizing these techniques, Purpura and Grundfest[9] have demonstrated that *d*-tubocurarine chloride (2 to 3 mg./kg.) produces a reversible blockade of the surface-negative wave evoked in a variety of ways. This phenomenon is illustrated in FIGURE 1. In FIGURE 1A, 3 mg./kg. *d*-tubocurarine reduced, and then abolished (FIGURE 1A3) the surface negative wave 1 minute after injection. This effect was reversed in 5 minutes (FIGURE 1A4), with almost complete recovery at the end of 20 minutes (FIGURE 1A5). Subsurface (0.8 mm. below the pia mater) stimulation revealed a similar train of events. The diphasic positive-negative response evoked from the motor cortex by subsurface stimulation (FIGURE 1B) showed loss of the negative component by 2 mg./kg. of *d*-tubocurarine within 45 seconds after injection (FIGURE 1B2), and almost complete recovery within 2 minutes (FIGURE 1B3).

Of particular interest was the demonstration that the surface negative wave evoked by antidromic stimulation of the medullary pyramids was also reversibly blocked by *d*-tubocurarine. In FIGURE 1C, the upper 3 series of tracings represent the antidromic cortical response to pyramidal stimulation, whereas below these are the orthodromic responses recorded from the same pyramidal-tract site following stimulation of the motor cortex. Analysis of orthodromic pyramidal-tract responses by Patton and Amassian[10] has indicated that the initial fast response is attributed to direct excitation of cell bodies or axons by the

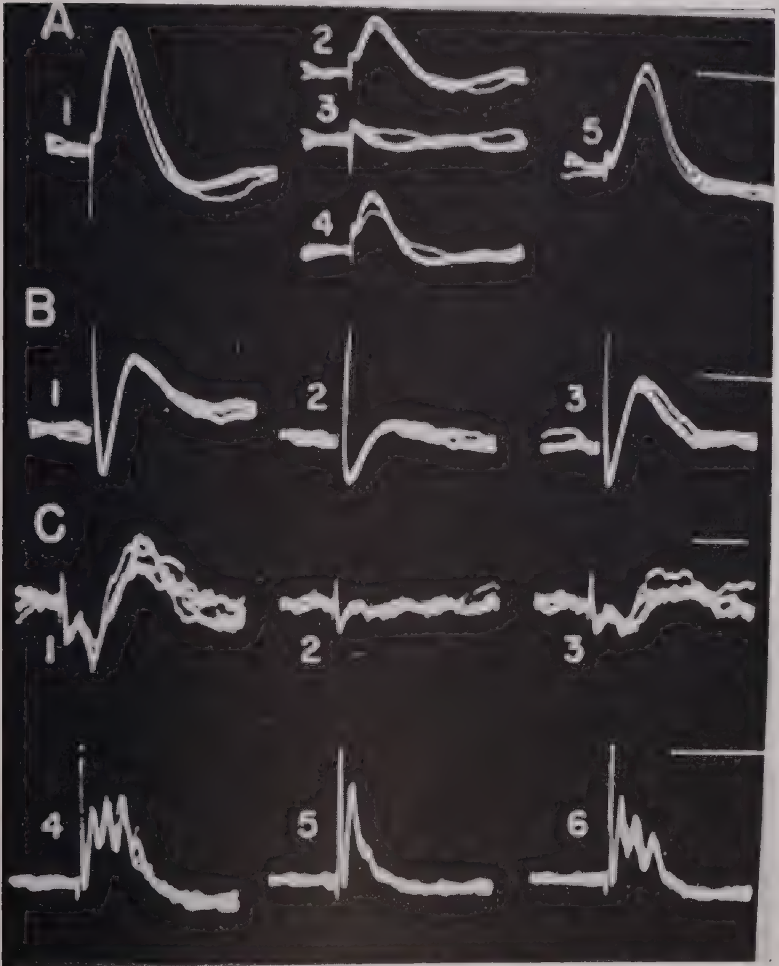

FIGURE 1. A. The dendritic potential (surface-negative wave) elicited by a cortical stimulus, recorded monopolarly 2 mm. from the site of stimulation. A1 = time of intravenous injection of 3 mg./kg. *d*-tubocurarine; A2 = 20 seconds after injection; A3 = 1 minute after injection; A4 = 5 minutes after injection; A5 = 20 minutes after injection, showing almost complete recovery (time: 20 msec.).

B. Subsurface stimulation of motor cortex by an electrode 0.8 mm. below pial surface (surface recording). B1 = control; B2 = 45 seconds after intravenous injection of 2 mg./kg. *d*-tubocurarine (the dendritic potential has been obliterated); B3 = almost complete recovery 2 minutes later. Note the lack of effect on the amplitude of the initial positive component (time: 15 msec.).

C. C1 to C3 = antidromic cortical response to pyramidal stimulation; C4 to C6 = orthodromic pyramidal response to the stimulation of the motor cortex site of antidromic registration; C2 and C5 show the responses 5 minutes after the intravenous injection of 3 mg./kg. *d*-tubocurarine. Note that the loss of the second and third spikes in the orthodromic responses correlates with the abolition of the second positive spike and the antidromic negative wave (C2). C3 to C6 = recovery 20 minutes after injection (time is 10 msec. for each). Multiple-sweep recordings in this figure and all subsequent records from Purpura and Grundfest.[9]

cortical stimulus (direct response), and all subsequent spikes at 2 to 2.5-msec. intervals is attributable to interneuronal bombardment of the pyramidal neurons (indirect responses). This interpretation is consistent with the observation that all responses other than the directly relayed activity are synaptically generated, as demonstrated by the inhibition produced by *d*-tubocurarine. Comparison of the orthodromic with antidromic responses recorded at the height of the central synaptic blockade (3 mg. kg. *d*-tubocurarine) indicates that the surface negative wave, as well as the second brief positive spike in the antidromic record, is of synaptic origin.

Since a more detailed account of the nature of the dendritic potential has been presented elsewhere (Purpura and Grundfest[11]) it is sufficient to record the 2 main conclusions that were derived from these experiments:

(1) The surface negative wave of Adrian[12] evoked by "direct" or antidromic stimulation of cortical neurons is a postsynaptic potential of the dendrites.

(2) The failure to detect any response attributable to dendritic activity at the height of the synaptic inhibition indicates that the dendrites are electrically inexcitable.[13] A more detailed account of the concept of electrical inexcitability of the synapse has been presented by Grundfest[14] elsewhere in this volume.

As a corollary to this it must be added for the purposes of the present analysis that the dendritic (synaptic) potential only can be generated by transmitter (neurohumoral) action, thus establishing more concretely the exclusively synaptic action of LSD on dendritic activity. Also established by this analysis is the fact that the oscillations of potential recorded from the cortical surface attributed to the summated activity of the dendrites[4-6] is entirely synaptic in origin. This is demonstrated in FIGURE 2, which shows that 2 minutes after the administration of 2 mg./kg. *d*-tubocurarine the EEG was completely abolished, but it returned at the end of 5 minutes and was normal 10 minutes after the injection. Similar results were obtained originally by Ostow and Garcia.[15]

Clarification of the synaptic nature of dendritic activity next poses the problem of demonstrating the presence of inhibitory as well as excitatory synapses on dendrites if the proposition is to be maintained that LSD may block the excitatory synapses or facilitate inhibitory dendritic synapses.

Inhibitory Afferent Pathways on Apical Dendrites: The Mechanism of Reticulocortical Activation

It has been inferred from a consideration of the form and distribution of evoked cortical potentials that, whereas afferents from specific-relay nuclei terminate in close relation to the cell bodies of cortical neurons (axosomatic synapse), afferents from intercortical, corticocortical, and "diffuse" or unspecific thalamic and brainstem systems terminate particularly on cortical dendrites (axodendritic synapses)[1, 4, 5, 7, 16, 17] or cortical elements other than those activated directly over specific pathways.[18-20] This inference has been extended to include the observation that the alteration in the spontaneous electrocortical activity produced by high-frequency stimulation of the bulbar reticular system is primarily associated with an inhibition of evoked dendritic activity (Purpura[21]). This inhibitory effect of brainstem stimulation on dendritic responses is shown in FIGURE 3. In this instance the electrocortical activity and tested

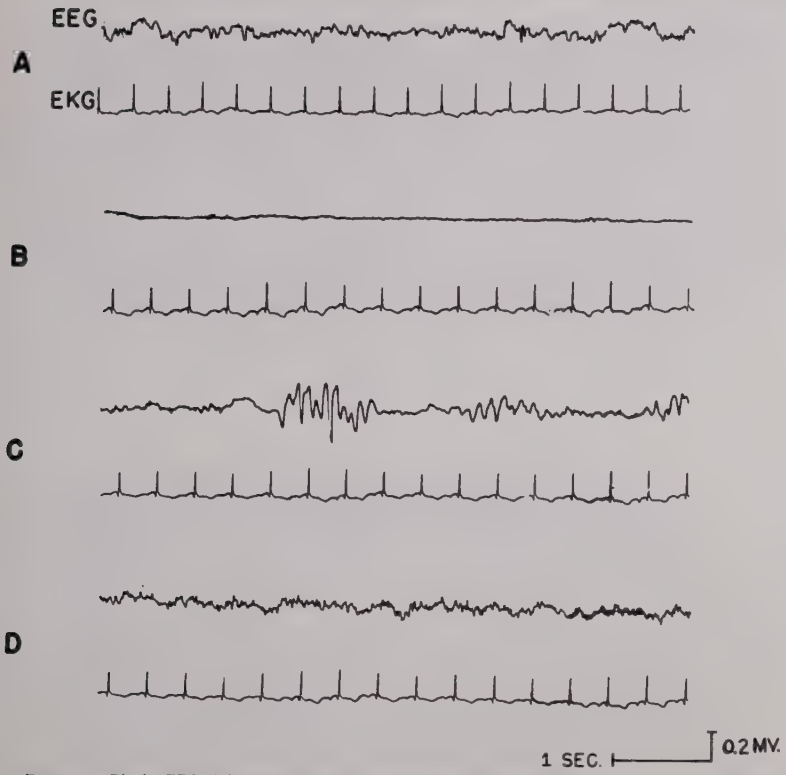

FIGURE 2. Bipolar EEG of the anterior sigmoid anterior suprasylvian gyrus of an unanesthetized cat treated with succinylcholine. A = control; B = 2 minutes after the intravenous injection of 2 mg./kg. *d*-tubocurarine; C = 5 minutes after injection; D = 10 minutes after injection.
Note the abolition of the EEG in B, unassociated with change in the EKG.

dendritic potentials were observed before, during, and after high-frequency (300/sec.) stimulation of the brainstem. It is of particular interest to note that the dendritic inhibition persisted along with the cortical activation for seconds after cessation of the stimulus.

Appreciation of the long-persisting alteration in dendritic synaptic activity following a short period of stimulation of the brainstem at the posterior tegmental level is shown in FIGURE 4. Construction of this figure was accomplished by placing successive oscilloscopic tracings side by side before, during, and after a 6-second stimulation of the brainstem. Responses were evoked every 2 seconds from the anterior suprasylvian gyrus. Viewed in this manner, an unsuspected order of complexity was noted in the recovery of dendritic activity from inhibitory afferent stimulation. In the 3-minute continuous run shown in FIGURE 4 the first stimulus of the brainstem (300/sec.) revealed an initial slow recovery followed by the development of a cyclic shortening of the response and diphasicity, with return to control amplitude at the end of 60

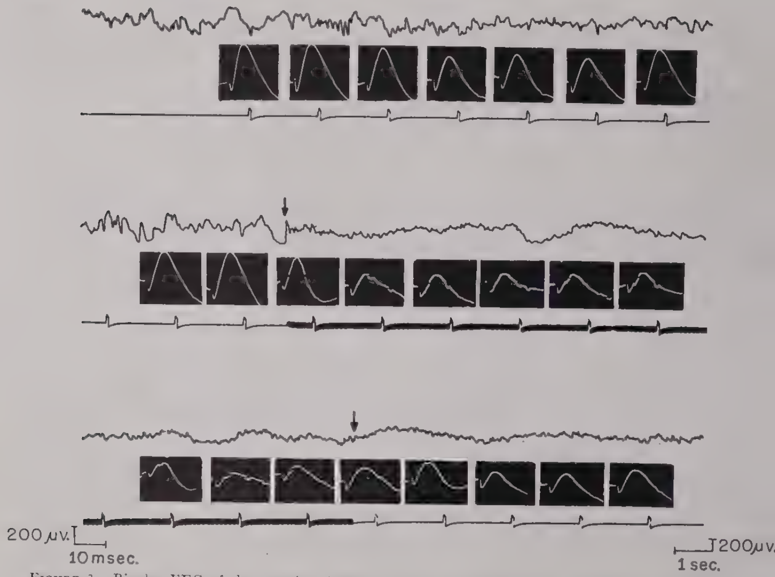

FIGURE 3. Bipolar EEG of the anterior sigmoid anterior suprasylvian gyrus. The dendritic potential is evoked by surface stimulation at the times indicated in the marker channel below the oscilloscopic tracings. The first arrow shows the onset of reticular stimulation of the upper pons, and the second arrow indicates the end of stimulation of the brainstem. The inhibition of the dendritic activity initiated by the stimulus is maintained beyond the period of the stimulus. The calibration at the left shows the oscillographic responses, and the calibration at the right shows the EEG.

seconds, only to develop the identical pattern again following another 6-second stimulation (FIGURE 4, B and C).

Although it is beyond the scope of this present report to attempt an explanation of the inhibitory mechanism involved, it is concluded from these observations that reticulocortical afferents exert inhibitory effects on dendritic activity either directly through transmitter action on inhibitory postsynaptic dendritic membranes or through indirect excitation of inhibitory cortical neurons similar in some respects to inhibitory (Renshaw) cells in the spinal cord.[22, 23]

That the inhibitory effect of reticular stimulation on dendritic activity is mediated by a discrete inhibitory system markedly sensitive to barbiturates at cortical levels as well as brainstem levels can be seen in FIGURE 5. In this experiment 10 mg./kg. of pentobarbital sodium, injected intravenously, was sufficient to depress slightly the excitatory cortical elements giving rise to the tested dendritic synaptic potential. The inhibitory effect of reticular (tegmental) stimulation, however, was almost entirely abolished by the barbiturate (FIGURE 5B). Indeed, it was often observed that the tested dendritic potential was *augmented* immediately following stimulation of the brainstem when the subanesthetic barbiturate had effectively blockaded the inhibitory action. It is of interest to note that the "anti-inhibitory" action of the barbiturates on evoked potentials was suggested by Brazier[24] in her analysis of the Forbes

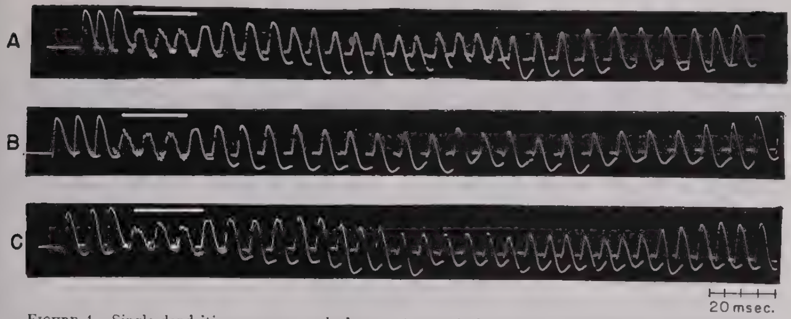

FIGURE 4. Single dendritic responses evoked every 2 seconds from the anterior suprasylvian gyrus. The white lines above the fourth, fifth, and sixth responses in A, B, and C indicate the duration of the stimulation of the brain stem in the post-tegmental region. The record shows a continuous series of 3-minute duration. Note the complex change in the evoked dendritic response after each stimulation of the brainstem, with recovery occurring at about 1 minute after the stimulus in A, B, and C.

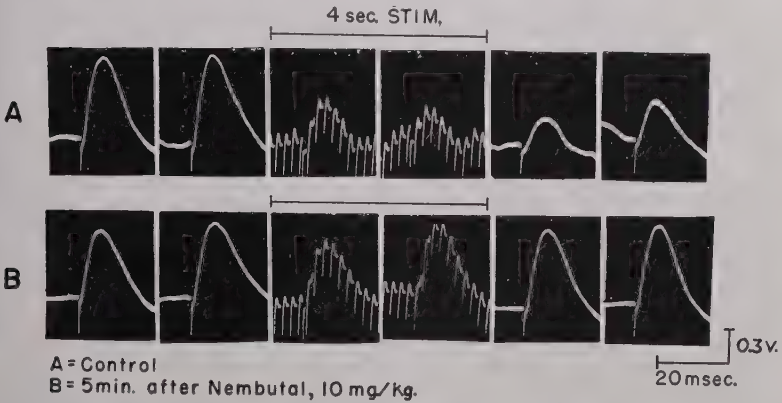

FIGURE 5. The anti-inhibitory action of pentobarbital sodium. This figure shows the dendritic potential evoked from the anterior suprasylvian gyrus 1 mm. from the cortical site of stimulation.

"secondary discharge." Consideration of the blockade of "tonic" inhibition produced by certain reticulocortical elements following the administration of small amounts of barbiturates may explain the paradoxical augmentation in both the amplitude and the projection area of evoked primary responses when these are studied in transition from the unanesthetized state to the barbiturized state. This explanation is contrary to the one proposed earlier by Bremer and Bonnet,[25] who have thought of this phenomenon in terms of "disocclusion." It should be clearly understood, however, that the anti-inhibitory action of pentobarbital sodium is not demonstrable even at very low concentrations without some depression of the excitatory elements. That there is a predilection for ascending reticular (inhibitory) blockade at low concentrations, nevertheless, already has been demonstrated.[26]

That it is not necessary to utilize repetitive stimulation of the brainstem to

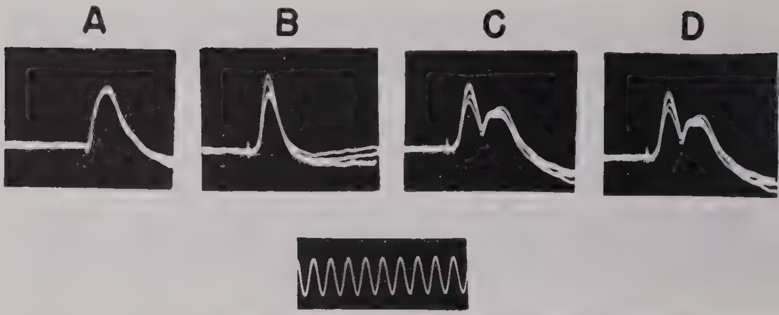

Figure 6. A = 5 consecutive superimposed sweeps, showing dendritic potential of the anterior suprasylvian gyrus, 2 mm. from the site of the cortical stimulus; B = the dendritic responses evoked from the same site by a single weak shock to the post-tegmental reticular system; C = inhibition of the evoked cortical response when stimulation of the brainstem precedes it by 20 msec. and 22 msec.

The calibration is 0.3 mv., 100 cycles per second. Multiple-sweep recording.

activate inhibitory elements on cortical dendrites is demonstrated by the predominantly inhibitory interaction produced by surface activation of the dendritic potentials and the activation of the same dendritic elements produced by stimulation of discrete parts of the bulbotegmental reticular system. In the unanesthetized animal a single-shock stimulation of the posterior tegmental regions evokes an exclusively surface-negative potential that is maximal in the anterior-superior suprasylvian gyrus. In more rostral cortical areas this response is initially surface-positive, which probably indicates a major difference in the mode of termination of the tegmentocortical afferents in the sensorimotor regions as opposed to the "association" cortex of the suprasylvian gyrus. The fact that subcortical afferents to *certain* cortical areas evoke specifically dendritic potentials confirms and extends the observations of Clare and Bishop[4, 5] that pathways activating only apical dendrites are related to "associative" and integrating systems.[16, 17] Although a more detailed account of the cortical distribution of potentials evoked from certain parts of the "ascending activating system"[27] will be presented elsewhere,[28] it is of interest to note (FIGURE 6) that marked inhibition of the tested surface response occurred following stimulation of the brainstem. That this was not simple refractoriness was indicated by the fact that when both responses were elicited simultaneously, with respect to the rising phase of both responses, addition occurred (not shown in FIGURE 6). Of particular interest also was the observation that during the inhibition of the tested surface response the simple negative potential decomposed into 2 independent deflections, revealing some of the components observed in the alterations in dendritic responses noted during recovery from high-frequency stimulation of the brainstem (FIGURE 4).

Although it is recognized that the final proof for the existence of inhibitory synapses on cortical neurons must await intracellular recordings similar to those obtained from spinal motoneurons[29] the foregoing data are considered more than suggestive evidence of the existence of inhibitory synapses on cortical dendritic elements. With this in mind a further analysis of the inhibitory action of LSD is now possible.

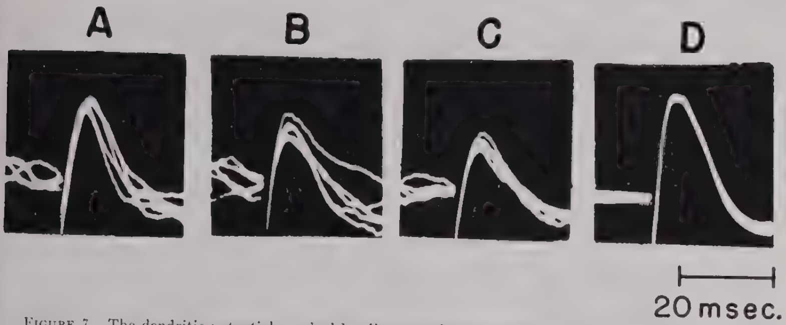

FIGURE 7. The dendritic potentials evoked by direct cortical stimulation. A = control; B = 20 minutes after the intravenous injection of 10 μg./kg. of LSD; C = 20 minutes after the intravenous injection of serotonin; D = 5 minutes after the subsequent intravenous injection of 10 mg./kg. of pentobarbital sodium. Multiple-sweep recording.

Inhibitory Nature of the Action of LSD on Dendritic Activity

Utilization of the 15- to 20-msec. surface-negative wave evoked by stimulation of the cortical surface or antidromic activation of cortical neurons as an example of relatively "pure" dendritic activity has been described above. The effect of LSD on these responses is presented in FIGURES 7 and 8. In FIGURE 7 it can be seen that reduction of the dendritic potential evoked by surface stimulation followed the intravenous injection of 40 μg./kg. of LSD (FIGURE 7B). This potential was further reduced by subsequent injection of 10 μg./kg. of serotonin (FIGURE 7C), and it was augmented markedly after 10 mg. kg. of pentobarbital sodium. Although it is beyond the scope of this paper to present evidence on the interaction of LSD and serotonin, it must be pointed out that in a series of neurophysiological investigations in progress no evidence of antagonism between these 2 compounds has been demonstrable on central synaptic activity (Purpura, Kandel, and Gestring[30]). On the contrary, whenever tested, LSD and serotonin acted additively, producing inhibition of dendritic activity similar in many respects to that of LSD, as was originally demonstrated by Marrazzi and Hart.[31]

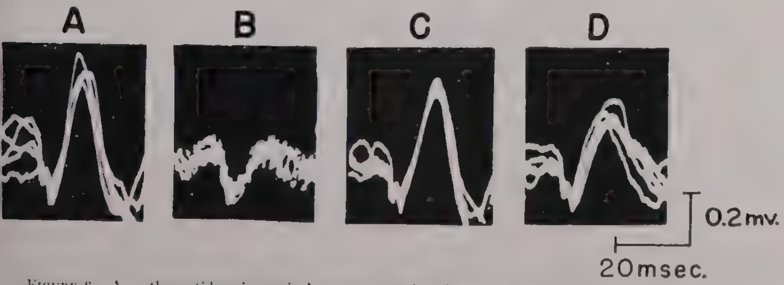

FIGURE 8. A = the antidromic cortical responses evoked from the motor cortex (anterior sigmoid gyrus) following the stimulation of the medullary pyramid at the mid-olivary region; B = the obliteration of the dendritic component by reticular stimulation of 8 seconds duration; C = recovery 30 seconds later; D = the inhibition of dendritic response 20 minutes after the intravenous injection of 40 μg./kg. of LSD. Multiple-sweep recording.

Comparison of the inhibitory effect of stimulation of the brainstem on dendritic activity evoked by antidromic pyramidal stimulation with the inhibitory action of LSD on this synaptic potential is shown in FIGURE 8. In this instance the inhibition produced by high-frequency bulbar reticular stimulation completely obliterated the dendritic potential in a manner similar to that observed on the dendritic response to surface stimulation (FIGURES 3 and 4). Following the administration of 40 μg./kg. of LSD, marked reduction in the dendritic potential occurred, with minimal effect on the initial brief positive components (FIGURE 8D). This effect may be attributed to the fact that the second surface-positive component is probably synaptic in origin, as previously indicated. It is of importance, however, to point out that reduction in the initial surface-positive spike in the antidromic cortical response to pyramidal stimulation, representing the invasion of axon and, presumably, cell body may be due to a generalized decrease in cellular excitability that is secondary to the inhibitory effect of stimulation of the brainstem and LSD. This effect is demonstrated by the marked change in direct cellular excitability during and immediately after reticular stimulation or the administration of LSD, as shown in FIGURE 9. In this composite of 3 different experiments, bulbar reticular stimulation inhibited the direct response recorded in the pyramidal tract to stimulation of the motor cortex during (FIGURE 9A) and for some time after stimulation (FIGURE 9, B and C, two other experiments). Similar effects were obtained (FIGURE 9D) in an experiment after the administration of 50 μg./kg. of LSD.

The effects of bulbar reticular stimulation on the direct cellular excitability may serve to explain the observations of the many investigators who have noted the reduction or abolition of resting-unit pyramidal discharge following physiological arousal (Adrian and Moruzzi[32]) or electrical stimulation of the ascending activating area (Calma and Arduini[33]). Although this phenomenon has been interpreted differently in the past, it would appear from the present investigation that inhibition of dendritic activity is associated in some way with a decrease in cellular excitability. Whether this presents a decrease in cellular excitability produced by hyperpolarization of dendritic elements or excitation of inhibitory synapses on pyramidal-cell bodies cannot be determined at this time.

Concerning the mechanism of the action of LSD on dendritic activity it seems reasonable to propose that this inhibition is more related to an excitation of inhibitory neurons or inhibitory postsynaptic receptor sites than to a blockade of excitatory synaptic activity. The reason for suggesting an increase in inhibitory activity on dendritic elements derives from 2 observations cited above: (1) the effect of LSD on dendritic activity mimics in many ways the inhibitory effect of stimulation of the brainstem; that is, the same inhibitory elements affecting dendritic synapses that are activated by reticulocortical afferents are available to the selective action of LSD; and (2) both effects are eliminated by the anti-inhibitory action of small amounts of pentobarbital sodium. If the action of LSD was to depress excitatory synaptic activity it does not seem probable that the augmented response produced by the barbiturate could be explicable on the basis of a direct excitation of the depressed elements. Since it has still to be demonstrated that pentobarbital sodium

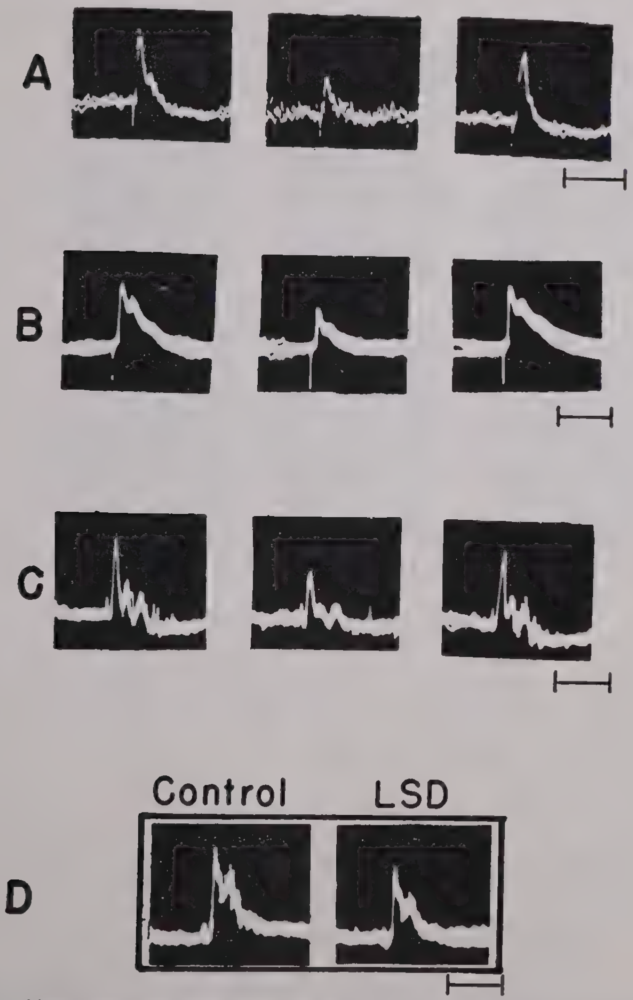

FIGURE 9. Three different experiments showing the effect of bulbar reticular stimulation (contralateral to the pyramidal recording) on orthodromic responses to bipolar stimulation of the precruciate cortex (anterior sigmoid gyrus). Line A shows the marked reduction during 300 sec. stimulation of the brainstem (middle series), with recovery 1 minute later being shown at the right. B and C show, at the left, the control responses; in the middle are shown the records after 10-second stimulus to the reticular system; and at the right is shown the recovery in each case 1 minute later.

D records the same experiment as C, but with a stronger stimulus to the cortex and 20 minutes after the intravenous injection of 50 μg./kg. of LSD (time: 10 msec.). Multiple-sweep recording.

facilitates any type of synaptic transmission, which is unlikely, the "paradoxical" augmentation of depressed dendritic activity produced by LSD or serotonin is believed to be related to a more selective blockade of inhibitory dendritic activity.

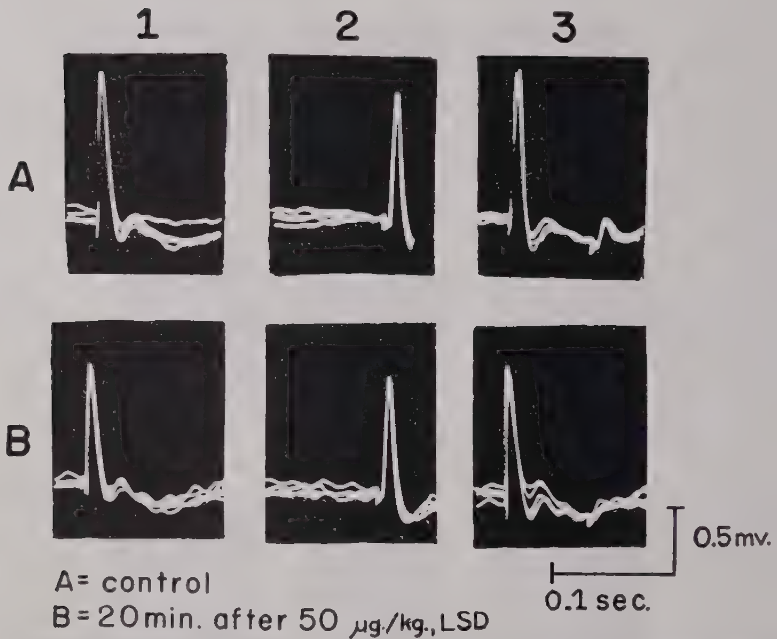

FIGURE 10. A1 shows the dendritic potential evoked by direct cortical stimulation of the anterior (superior) suprasylvian gyrus. A2 shows the dendritic potential evoked by single-shock stimulation of the posterior tegmentum at the same cortical site as A1. A3 shows the inhibitory interaction when cortical stimulus precedes brainstem stimulus by 65 msec. Note the almost 90 per cent inhibition of the reticulocortical response during the "after-positive" potential that developed after dendritic response to cortical stimulation.

B shows the same sequence after the intravenous injection of 50 μg./kg. of LSD. Note that, despite the reduction of both responses, an almost inhibitory interaction developed after the administration of LSD. Multiple-sweep recording.

That the mechanism of action of LSD appears to involve an active increase in dendritic inhibition by the excitation of inhibitory cortical interneurons or the stimulation of inhibitory receptor sites on apical dendrites is consistent with observations of the effect of LSD on inhibitory interaction of cortical and brainstem stimulation (FIGURE 10). From this study it can be seen that the marked inhibition of dendritic activity evoked by tegmental stimulation in the association cortex of the anterior suprasylvian gyrus following a prior surface stimulus is almost complete after the administration of 50 μg./kg. of LSD. FIGURE 10 emphasizes the prolonged inhibition set up on dendritic elements by the prior cortical stimulus which, 65 msec. later, still reduced the reticulocortical response by 90 per cent. Of more than incidental interest is the fact that this inhibition is superimposed on the slow "after-positive" component of the dendritic response.

Implicit in the hypothesis that LSD activates inhibitory dendritic elements also available to afferents from certain reticular regions of the brain stem is the assumption, made mandatory by the exclusively synaptic nature of the dendritic potential, that reticular activity is translated on cortical dendrites through the liberation of transmitter substances. Acceptance of this hypothesis thus requires, in part, presentation of satisfactory evidence in favor of the existence of transmitter (neurohumoral) agents at some stage of reticulocortical activation.

Neurohumoral Reticulocortical Activation

Although theories relating to the existence of neurohumoral agents affecting brain activity date back to the time of Hippocrates, it was Ingvar[34] who first suggested that the alterations observed in slabs of neuronally isolated cortex produced by reticular stimulation might be mediated by means of extraneuronal factors. That the long-persisting alterations of dendritic activity observed by reticular stimulation might involve the accumulation of a humoral agent or agents at the brainstem and cortical dendritic synapses rather than a continuous re-excitation in closed neural circuits was also suspected from data relating to the effects of reticular stimulation on strychnine and metrazol spikes evoked by topical application of these 2 convulsants.[35] An example of this is shown in FIGURE 11. In this experiment 300 cycles per second stimulation of the bulbar reticular formation at the point shown in the brainstem section below the tracings produced usual "activation" of the cortex (left anterior sigmoid to anterior suprasylvian leads) that persisted for seconds after the cessation of the stimulus (FIGURE 11A). Following the application of pledgets of 1:2000 strychnine to electrode sites, a short stimulus to the brainstem (FIGURE 11B) activated 3/second spikes from the cortex with a latency of about 12 to 15 seconds after the onset of the stimulus. More prolonged stimulation (FIGURE 11C) minimally shortened this latency, but intensified the 3/second spike and "spike-wave" cortical activity. It should be pointed out that in this experiment the strychnine alone was below the threshold for the activation of cortical spikes. Although the mechanism of facilitation and inhibition of topically evoked strychnine activity remains enigmatic despite attempts at clarification,[36] these results indicated the necessity for a further consideration of extraneuronal influences of brainstem stimulation on cortical activity.

To investigate these influences, experiments were performed on pairs of unanesthetized intact and *encéphale isolé* cats. Animals were prepared as shown in FIGURE 12, with cannulation of both femoral arteries and veins to permit rapid cross-circulation. This technique and its limitations have been described more fully elsewhere.[8]

To summarize the results briefly, it was shown that stimulation of the bulbar reticular formation of one animal, the "donor," was capable of activating the cortex of the other animal, the "recipient," after a latency of from 30 to 80 seconds. In FIGURE 13 an example of this is presented that shows the electrocortical activity of the recipient animal only before (FIGURE 13, 1), after 40 seconds of stimulation of donor animal (FIGURE 13, 2), and 1 minute after cessation of the stimulus. Although the latency of activation varied in the range noted above in all experiments, in any single experiment this latency remained remarkably constant (±10 sec.).

That reticular stimulation of the donor animal was capable of humorally

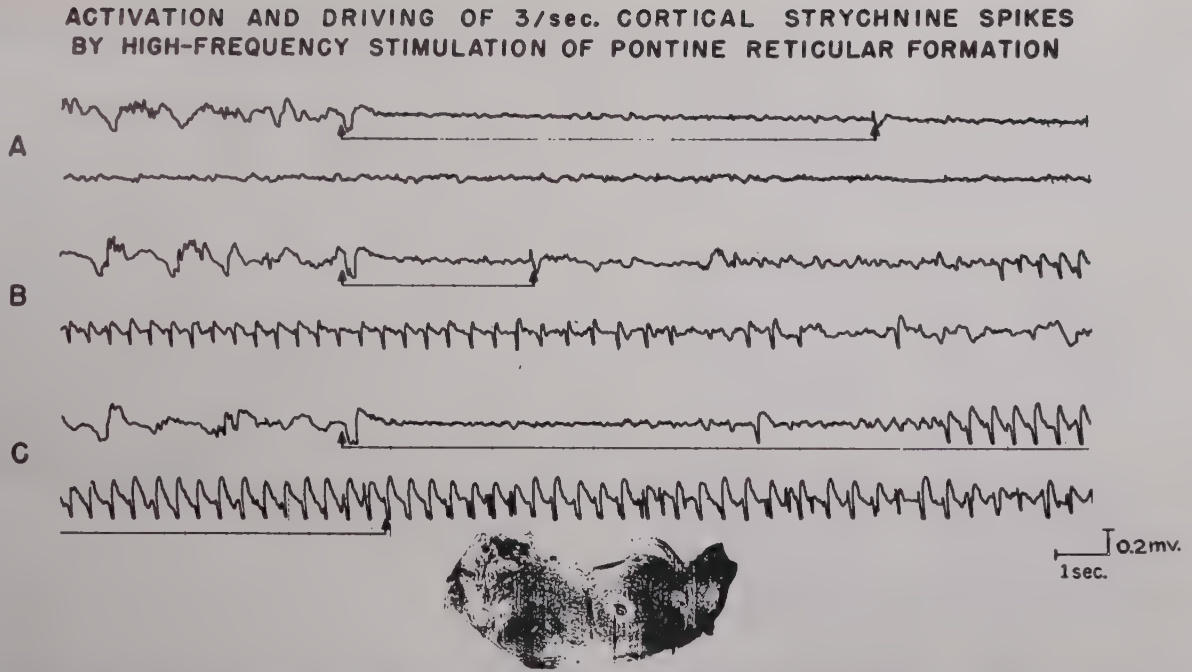

FIGURE 11. Bipolar EEG of the postsigmoid anterior marginal gyrus. In A, the control, a 10-second stimulus produced an activation pattern that persisted for seconds after the stimulus; in B, 10 minutes after topical 1:2000 strychnine, a short stimulus produced long-latency 3-second driving; in C, a longer stimulus exerted a more pronounced effect.

Below is shown a photomicrograph of the site of stimulation in the mid-pontine reticular location.

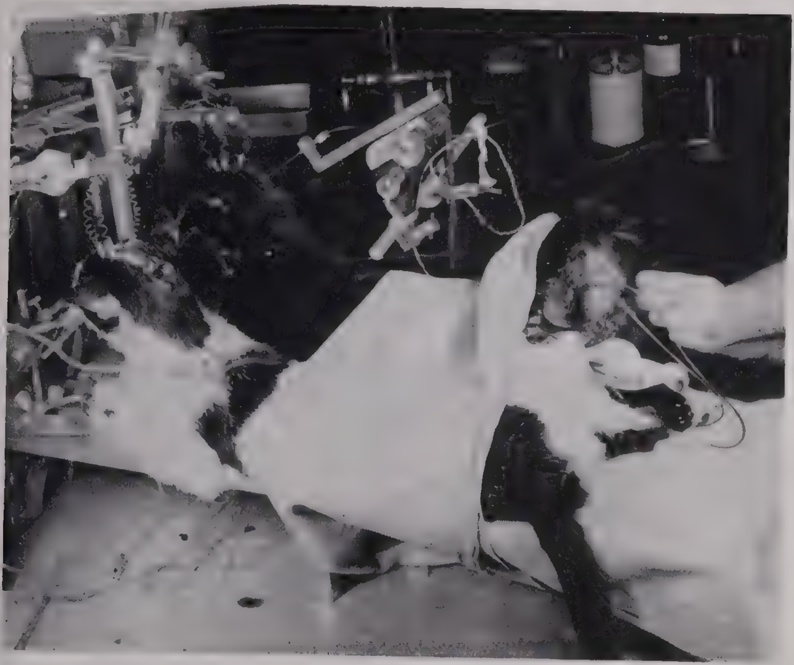

FIGURE 12. A photograph of the setup used for cross-perfusion experiments in pairs of cats. In this instance the stimulation of the brainstem was carried out on the black cat, but EEG's were recorded from both animals. Polyethylene cannulae are shown passing from the femoral arteries to the femoral veins of both animals.

facilitating metrazol spikes in the recipient animal in a manner similar to that shown in FIGURE 11 for the single preparation is demonstrated in FIGURES 14 and 15. In FIGURE 14, 1, the cortical activity of the recipient animal exhibited intermittent spikes after topical application of a few drops of 5 per cent metrazol. Approximately 30 seconds after the onset of bulbar reticular stimulation of the donor animal, facilitation of this spike-activity occurred, reaching maximal limits after about 70 to 80 seconds of stimulation. Two minutes after such stimulation (FIGURE 14, 3) this activity became intermittent again.

Another example of this effect in *encéphale isolé* cats is shown in FIGURE 15. The choice of these preparations was guided by the desire to avoid marked alterations in blood pressure in the donor animal caused by bulbar stimulation[37] affecting the hemodynamics of the recipient animal and producing activation of the latter by this mechanism.[38] In this instance 20 to 30 seconds of stimulation of the donor animal produced activation and driving of the metrazol-facilitated cortex of the recipient animal, but this effect disappeared 2 minutes following the end of bulbar reticular stimulation of the donor.

It is concluded from these studies that reticular stimulation of 1 animal releases from the brain of the stimulated, or donor, animal a neurohumoral

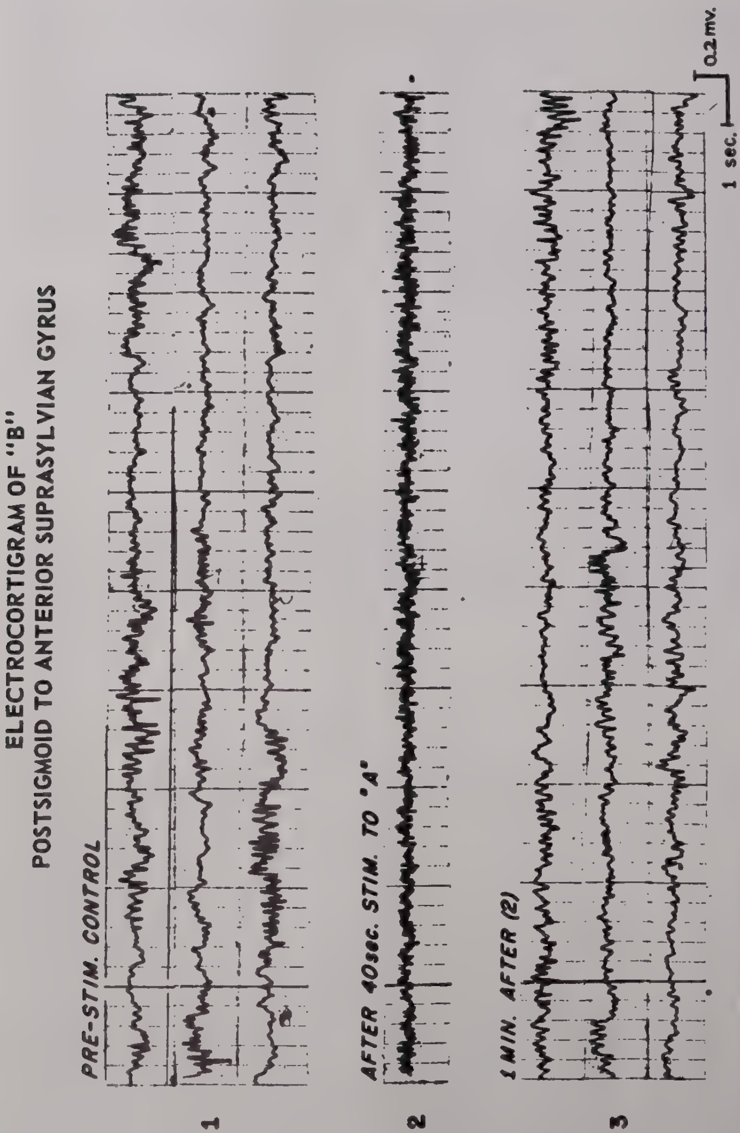

FIGURE 13. EEG of recipient "B" animal only: 1, a 1-minute continuous control run; 2, after bulbar stimulation of donor "A" animal; 3, poststimulation run of 1-minute duration, returning to resting activity.

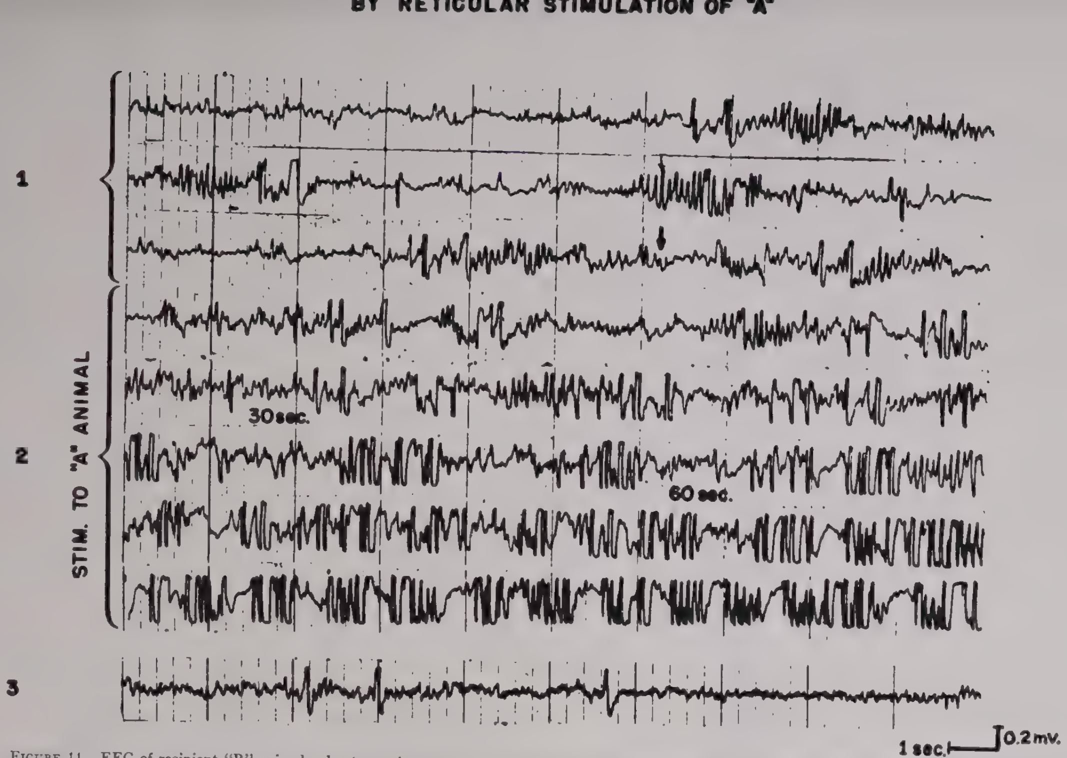

FIGURE 14. EEG of recipient "B" animal only: 1, continuous run 30 minutes after the application of a few drops of 5 per cent metrazol to the cortex of recipient (arrow in third line indicates the beginning of the stimulation of the brainstem of "A," the donor animal); 2, 60 seconds after the beginning of stimulation a marked augmentation of convulsant activity occurred; 3, this augmentation was reduced to intermittent activity 2 minutes after the end of stimulation. Note the persistence of the activation pattern during the interspike phases.

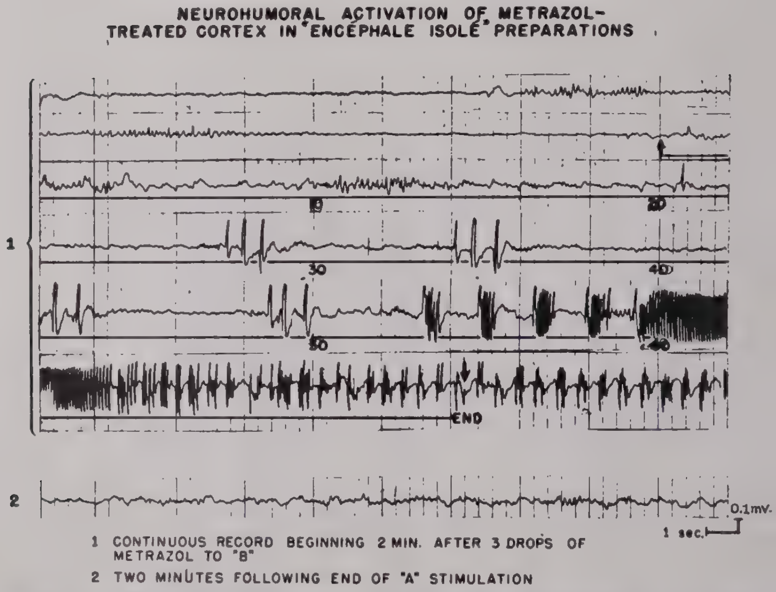

FIGURE 15. EEG of recipient "B" animal only: 1, arrow in second line shows stimulation of the brainstem o donor 2 minutes after the topical application of 5 per cent metrazol to the cortex of the recipient (augmentation to maximal sustained seizure occurred at the end of 60 seconds of stimulation, the end of which is shown by the second arrow, in the sixth line); 2, resting activity 2 minutes later.

factor (or factors) that, on being carried by way of the circulation to another (recipient) animal, is capable of reproducing all the features generally associated with physiological or electrical stimulation of the reticular "ascending activating system." While these observations point to the existence of transmitter substances affecting dendritic (as well as other cortical) elements normally liberated by reticulocortical activation, no further information is available at this time concerning the biochemical identity of these agents. It is inferred from the long latency and persisting effect that the agent or agents involved may be degraded slowly or liberated in the course of certain intermediate stages as yet undefined.[8]

DISCUSSION

In the light of the experimental data summarized above, further information is at hand to extend the original observations on the nature of the inhibition of axodendritic synaptic activity by LSD.[1] The hypothesis that dendrites are only synaptically excitable (through transmitter action), which follows from the failure to detect activity assignable to these elements after complete central synaptic blockade (Purpura and Grundfest[11]), coupled with the finding that brainstem afferents on repetitive stimulation exert marked inhibitory action on dendritic activity that mimics the action of LSD, suggests that the action of LSD is to activate inhibitory elements available also to reticulocortical afferents. With regard to the inhibitory action of brainstem stimulation on cortical dendrites, it is of interest to note that Sprague[39] has demonstrated anatomically a distribution of monosynaptic inhibitory dorsal-root collaterals exclusively on dendrites, whereas excitatory collaterals are distributed diffusely over the neuron. With all due precautions about translating the results obtained on spinal motoneurons to cortical neurons, this finding may explain why certain afferents synapsing exclusively on dendrites in some cortical areas exert long-lasting inhibitory effects on dendritic activity.

That the inhibition exerted at the dendritic level following stimulation of the brainstem produces a demonstrable decrease in cellular excitability as tested by the magnitude of the tested directly relayed pyramidal response is in contrast to the observations of Brookhart and Zanchetti.[40] Utilizing the "pyramidal preparation" of Whitlock,[41] these investigators were unable to demonstrate an alteration in corticospinal responsiveness during stimulation of rostral mesencephalic reticular regions capable of producing typical electrocortical activation. Since in the present report the effects on the excitability of pyramidal cells followed stimulation of bulbar rather than more rostral portions of the ascending activating system, this phenomenon may suggest a functional segregation within this system of prepotent regions mediating predominantly inhibitory activity on cortical dendrites. This conclusion has already been derived from experiments relating to the rostral limit in the brainstem at which a complete transection can be made without altering the electrographic pattern associated with the *encéphale isolé* preparation.[42]

Finally, the fact that transmitter agents can be demonstrated to play a role in the persisting alteration in dendritic responsiveness during electrocortical activation suggests the possibility that LSD may compete at the inhibitory receptor site on apical dendrites for the transmitter liberated by inhibitory cortical neurons or afferent terminals by reticular stimulation. If, as has been previously proposed and confirmed in the present investigation, dendritic activity generates the spontaneous electrocortical activity recorded from the surface of the brain, the "flattening" or activation pattern observed after the administration of LSD might be considered to be due to cortical (inhibitory) action of LSD competing for the same receptor activated by presynaptic reticulocortical afferents. It is concluded that the electrographic picture common to reticular stimulation and the action of LSD does not indicate a direct action of LSD on the brainstem reticular formation, but a diffuse effect on cortical as well as subcortical synapses whose activation will facilitate or inhibit transmission depending on whether this activation produces depolarization or hyperpolarization of the postsynaptic membrane.[14] It is within this structural framework that a more reasonable appraisal of the action of agents affecting the activity of the brain is to be sought. Further speculation at this time on the nature of the inhibition of axodendritic activity would exceed the present experimental data upon which it could be based.

While it is still unclear what information the psychotomimetic action of LSD will produce in permitting a more definitive answer to the problem of the etiology of "functional" psychoses, there can be little doubt that the use of this agent and the other hallucinogens as neuropharmacological tools has contributed much to the essential problem of "how the brain works." Tracing the historical development of neurophysiological concepts in epilepsy,[43] it is of interest to note that much of the present information on this subject originally was derived from attempts to analyze the mode of action of the neuropharmacological agents that depress consciousness. It may be anticipated that the use of psychopharmacological agents will play an analogous role in permitting a more complete understanding of the physiological basis of mind.

Summary

Further analysis of the inhibitory action of LSD on dendritic activity has been presented. It has been demonstrated that dendrites are only synaptically excitable and cannot be activated by direct electrical stimulation or by antidromic propagation from cell bodies. This finding indicates a synaptic site of action of LSD and that, particularly in the case of apical dendrites, it is an inhibitory action. Rather than being related to a blockade of excitatory synapses, it is proposed that LSD activates inhibitory synapses on dendrites that are also available to the transmitter or transmitters that are released by reticular stimulation of the brainstem. The existence of the latter phenomenon has been demonstrated by cross-perfusion experiments in unanesthetized cats.

Viewed from the cortical surface and neglecting the dynamics involved, the net result of the inhibitory action of LSD would appear to be an EEG pattern resembling that generally associated with behavioral arousal. That this may represent a competitive type of blockade of reticulocortical synapses points out the difficulty of attempting to interpret the mode of action or possible behavioral effects of psychopharmacological agents from the gross electrical activity of the entire cerebrum.

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GENERAL PROBLEMS OF DRUG ACTIONS ON BIOELECTRIC PHENOMENA*

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Introduction

The problem of synaptic transmission has recently been sharpened by new data and concepts. At all synaptic junctions studied thus far, including several of them in the mammalian central nervous system, postsynaptic potentials (PSP) are observed that develop in the postjunctional cell and have a form different from that of the spike of nerve or muscle. The response may have the same sign as the spike (depolarizing excitatory PSP) or the opposite (hyperpolarizing inhibitory PSP), and its duration is usually longer. In all cases examined for this, the PSP also appears to have a different mode of electrogenesis from that which causes the spike. This mode is evoked by neural stimuli or by chemicals, but not by direct electrical excitation; it behaves differently from the spike under various electrochemical conditions; and it is capable of manifesting itself while the spike-generating membrane is inactivated. On the other hand, the PSP can be eliminated selectively, while electrically excitable membrane still remains electrogenic. This and other evidence have led to the suggestion[1-6] that the postsynaptic membrane in all junctional systems has fundamentally different properties from the spike-generating membrane of the same cell and, specifically, that it is not electrically excitable, but that it responds only to various types of chemical stimuli. Some of the evidence will be described briefly below, and it will be seen that all cases of junctional transmission in which relevant data are available support this view. To the extent that this generalization applies, synaptic transmission is necessarily and obligatorily mediated chemically.

The recognition of fundamentally different properties in different electrogenic components of the same cell also permits a clearer analysis of, and provides some reasonable explanations for, various types of drug effects at synapses. In this account of synaptic pharmacology, the general thesis is that synaptic drugs exert their actions either by exciting or by inactivating the electrogenesis of postsynaptic membranes. These effects in turn play upon the electrically excitable component of the membrane. The combined results determine the experimentally observed overt actions of the drugs in various synaptic systems. This view does not preclude the current tendency to ascribe drug actions to effects upon one or another enzyme system of living cells. Nevertheless, and although such effects may represent the fundamental actions of drugs at molecular levels, the point of view brought forward here, emphasizing the electrogenic aspects of these effects, has the virtue of being amenable to immediate experimental observations and tests. Furthermore, as I shall endeavor to show, this approach has achieved considerable success in formulating and predicting experimental situations, as well as in encompassing a wide variety of synaptic systems and drug actions.

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An important corollary of this view is that drug actions must be considered, in each specific instance, in relation to the particular electrogenic structures and functions of the systems under study. The nature of the postsynaptic membrane determines its sensitivity to drugs, as well as the type of its electrogenesis. The relations of different types of junctional membrane that may be present in a given cell determine the net synaptic electrogenesis and its transfer to the conductile mechanism of the electrically excitable spike-generating membrane. The kinetics of the electrogenic phenomena of the latter and their actions in transferring excitation either to a subsequent postsynaptic element or to contractile or secretory mechanisms lead to the overt effects that are usually observed in the over-all responses of a given system or of the whole organism.

Thus anatomical and functional considerations enter prominently into this analysis of drug actions. A brief summary of the fundamental properties of different electrogenic mechanisms and of their interactions will therefore begin this account. Then will follow an analysis of the pharmacology of peripheral synapses, in terms of their increasing degree of anatomical and functional complexity. This discussion will be based chiefly on those systems that are best known electrophysiologically and pharmacologically: electric organs, skeletal muscle, and autonomic ganglia. The concepts will also be applied, however, to the large groups of autonomic effectors because the wealth of pharmacological data on these presents an interesting challenge, although the electrophysiological data as yet are scanty. The account will conclude with the consideration of synaptic pharmacology in the mammalian nervous system, first at the spinal level and then at the cortical level. Some recent experimental results in these fields obtained in collaboration with D. P. Purpura will serve to illustrate the analysis. Other data are presented by Purpura.[7]

The Different Modes of Electrogenesis

The Source of Bioelectric Potentials

Most and perhaps all cells maintain a different internal ionic composition than that existing in their surrounding fluids. This unequal distribution of ions results in an electrical polarization across the membrane of the cells and, in all cells so far known, the interior is negative to the exterior.[4, 8] The potential difference appears to be roughly related to an excess of internal K^+, approximately according to the equation

$$E = 58 \text{ mv. } \log \frac{K^+_i}{K^+_o}$$

In most cells examined, E, the resting potential, has a value ranging from about 50 to 100 mv.

The unequal distribution of K^+ is also associated with unequal but oppositely directed distributions of Na^+ and Cl^-, these ions being less concentrated in the interior of the cell. The ratios $\frac{Na^+_o}{Na^+_i}$ and $\frac{Cl^-_o}{Cl^-_i}$ usually are quite different from the reversed ratio $\frac{K^+_i}{K^+_o}$. A complex and as yet imperfectly understood series of metabolically energized processes transport Na^+ outward and K^+ inward across the cell membrane to establish the observed ionic asymmetries. The latter therefore do not represent a Donnan equilibrium, but a steady state condition of active transports.[9, 10]

The resulting electrochemical gradients constitute a source of potential energy that can develop bioelectric responses. The electrogenesis of activity, representing a change from the resting potential, results when the cell membrane, normally very highly resistive (specific resistivity of about 10^9 ohm-cm.),[4, 5] becomes more permeable in response to stimuli. Neither the nature of these changes nor their modes of action are known. Depending upon the specific type of excitable membrane and its electrochemical conditions, however, specific changes occur that cause a temporary flow of certain ions in the direction of their electrochemical gradients.[8, 11] The various types of electrogenesis in bioelectrically active postjunctional cells are shown in FIGURE 1.

The Generation of the Spike

Hodgkin and his colleagues[11] have developed a remarkably coherent analysis of the processes that evolve the spike of electrically excitable membrane. A small initial depolarization increases the permittivity of the membrane for both K^+ and Na^+, but the permittivity for the latter increases much more rapidly.

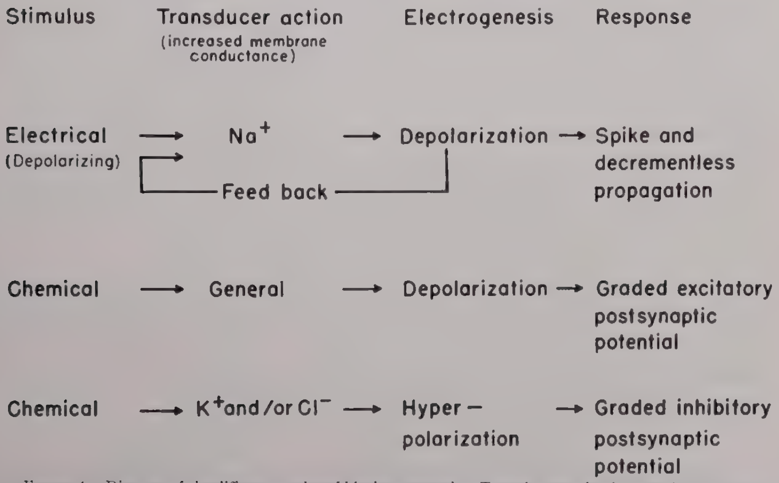

FIGURE 1. Diagram of the different modes of bioelectrogenesis. Transducer action is a membrane response to a stimulus that alters its permeability and causes the membrane potential to change from its resting value. Electrically excitable transducer membrane is found in conductile tissue: nerve cells and fibers, muscle fibers, and electroplax. Chemically excitable membrane occurs at the postjunctional surface of cells and probably also at receptor sites of muscle and sense organs. The latter may also have membrane specifically excited by thermal, photic, or mechanical stimuli.

Na^+ then enters from the more concentrated phase of the external medium, and these positive ions cause further depolarization of the cell. Sodium conductance rises with the decrease of the membrane potential. Sodium influx thus is regenerative, causing a change in membrane potential from approximately the level of the potassium equilibrium (E_K) to that of the reversed potential of the sodium equilibrium (E_{Na}). The relation describing this potential is

$$E_{Na} = 58 \text{ mv. } \log \frac{(Na_o)}{(Na_i)}$$

Since the ratio of sodium distribution is about 10:1, the new membrane potential is about 58 mv., inside positive. The response therefore constitutes a change from internal negativity during the resting potential to a positivity (or overshoot) during the spike, and a total excursion of about 100 to 150 mv. (FIGURE 2).

As the potential changes, however, the Na^+ transport of the membrane is decreased and stopped by a process of "sodium inactivation."[11] The K^+ flux is also increased by the changed membrane potential and is outward, since the depolarization and the reversal of membrane potential alter the electrochemical conditions for this ion. The outflow of these positive charges therefore dissipates the new membrane potential caused by the earlier influx of Na^+, restoring the initial state, although sometimes with an undershoot (FIGURE 2). The slower, metabolically energized processes of active transport then operate to eliminate the small amount of excess internal Na^+ and to rectify the corresponding deficiency of internal K^+.

The spike, rising from the resting potential to the peak of its overshoot, is an explosive *all-or-none response*, admirably suited to propagation, since the impulse is capable of decrementless conduction over the extension of the fibers in the nervous system or over muscle fibers. Under certain conditions either in normal or in poisoned cells, however, graded responses, capable only of decremental propagation are also produced.[1-5, 12-14] This behavior, although instructive as to the processes underlying electrical excitation, need not enter into discussion at present except to serve as an indication that graded electrical responsiveness is another form of bioelectrogenesis.

SYNAPTIC ELECTROGENESIS

Graded responsiveness is a characteristic of synaptic electrogenic activity. A more fundamental aspect, however, is the nature of synaptic excitation. A considerable body of evidence indicates that synaptically excitable postjunctional membrane does not respond to electrical stimuli.

Evidence for Electrical Inexcitability of Postsynaptic Membrane

(1) The first indication of this probably dates from Bernstein's discovery in 1882[15a] that neural excitation of muscle always involves a latency, whereas direct excitation elicits a response with a vanishingly small latency as the stimu-

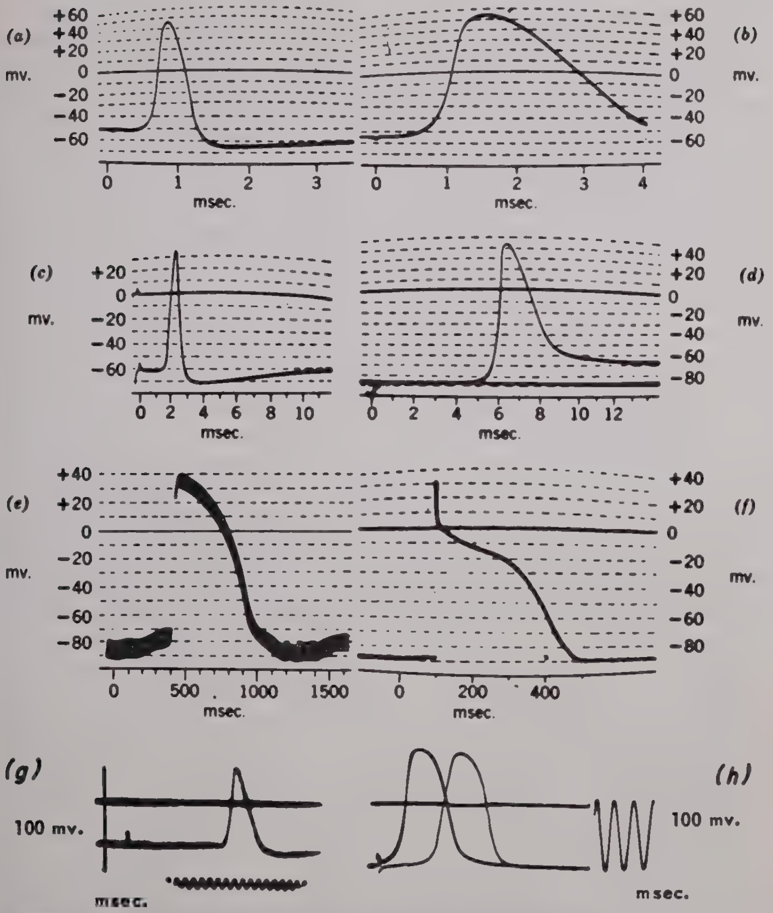

FIGURE 2. Spikes in different excitable cells.[9] (a and b) Giant axon of *Loligo* at 18.5° C. and 5° C. respectively; (c) giant axon of *Sepia*; (d) frog skeletal muscle fiber; (e) frog heart muscle; (f) dog Purkinje fibers; (g) tissue-cultured chick dorsal root ganglion cell; (h) directly stimulated electroplaque of *Electrophorus*. The response was recorded simultaneously in 2 regions of the cell.

lus strength is increased. Bernstein suggested that a special excitable tissue is interposed between the exciting nerve impulse and the effector muscle fibers.*

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* While Bernstein[15a] is generally credited with this finding (see Biedermann,[15b] p. 743) it seems to have been known in 1874 to du Bois-Reymond[15c] (p. 714) through the work of his student Sigmund Mayer. Du Bois-Reymond also cites the early history of the theory of electrical transmission first developed by Krause. As stated by Kuhne,[16] "a nerve throws a muscle into contraction only by means of its currents of action" (see also Gotch[17]). The electrical theory was reformulated by Lapicque[18] on the basis of isochronism, or the close similarity between the excitabilities of nerve and muscle to electrical stimulation. Heterochronism (loss of this similarity) resulting from drugs was supposed to be responsible for block of neuromuscular transmission. This theory was demonstrated to be incorrect in experiments with innervated single muscle fibers.[19] Keith Lucas[20] accepted Bernstein's

(2) Kuffler[25, 26] showed indirectly that the muscle end plate is not excitable by the action currents of the nerve. Fatt and Katz[27] made the same inference. Subsequent work has demonstrated that the end plate potential (EPP) is generated in a membrane component that is not electrically excitable,[28] but that responds to externally applied acetylcholine,[29, 30] even when the spike-generating membrane is inactivated.[31, 32]

(3) With intracellular recordings in various synaptic systems it has been noted that the change in voltage of the postjunctional cell caused by the current flow of the presynaptic impulse is not sufficient to excite the cell.[4, 5] In all of these, therefore, Krause's hypothesis appears to be invalid, and another mechanism is required to explain transmission.

(4) The "slow" muscle fibers of the frog[33] are not electrically excitable,[34] and the graded "junctional potentials" of neural stimulation are therefore EPP or PSP.

(5) Several types of invertebrate muscle fibers are likewise not excitable by electrical stimuli.[2, 35-38] These, as well as the frog "slow" fibers, are capable of sustained graded contractions in response to neural stimuli.

(6) In the 1890's, Gotch[17] and Garten[39] found that electrical excitation of electric organs of the Torpedines and of *Malapterurus* always caused the response after an irreducible latency. Both suggested that the organs were not electrically excitable. Garten also showed that denervated or curarized organs were not responsive to the strongest applied electrical stimuli. The organ of the *Torpedo* was subsequently found to be excitable by acetylcholine.[40]

(7) The above data were not incorporated into electrophysiological doctrine probably because of contradictory findings on the organs of the electric eel. When neural excitation of these is blocked by fatigue or by curarization, the organs still remain excitable to electrical stimuli.[41] The denervated organ is likewise electrically excitable.[42, 43] This discrepancy has been cleared up by intracellular recordings from single electroplax[2] that showed that the cells possess a dual excitatory mechanism (FIGURE 3). Stimulation of the nerves, and only of these, causes a small, early, graded, depolarizing response which is a PSP. The latter occurs always after an appreciable latency. It is not subject to refractoriness of the cell, nor is it blocked by strong hyperpolarization of the cell membrane as the spike is. The PSP is especially subject to "fatigue" of the repetitively stimulated nerve, but it also shows long-lasting facilitation (FIGURE 4). Its production is selectively blocked by the synapse-inactivating, nondepolarizing drugs (FIGURE 5 and TABLE 1). The PSP has different magnitudes at different regions of the cell membrane, but a depolarization of about 30 to 40 mv. initiates a spike, similar to that of axons or muscle fibers, which is propagated decrementlessly along the cell. The spike is also elicited by electrical stimulation and occurs even when the cell is denervated. Thus, the electrogenic behavior of the eel electroplaque is similar to that of its embryological relative, the muscle fiber. In the electroplaque, however, the contractile machinery of the muscle fiber has been dispensed with, and the sites

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view, and thought that the end plate was the seat of an electrically excitable component with special properties. Studies with single innervated muscle fibers could not demonstrate this component.[19, 21] The last comprehensive theory of electrical transmission[22] has since been abandoned.[23] The founder of electrophysiology, E. du Bois-Reymond[15b (p. 700)], appears to have been the first to suggest chemical transmission at the neuromuscular junction.[24]

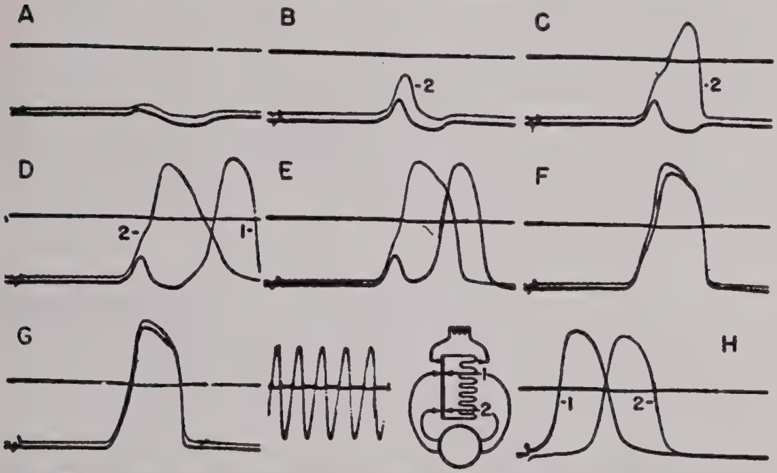

FIGURE 3. Neurally evoked responses in eel electroplaque.[2] The responses at 2 recording sites (1 and 2 of insert) are shown simultaneously. A weak stimulus to one of the nerve trunks supplying the cell produced simultaneously at both sites of the latter a small depolarizing potential (*A*), which increased (*B*) with a stronger stimulus to the nerve (the hyperpolarizing second phase is an artifact due to activity of other cells). Progressively stronger stimulation of the nerve caused a spikelike response at site 2, where the initial depolarization is largest. A spike developed subsequently (*D*) and propagated to recording site 1, arising there after a local response. Still stronger stimuli caused this region to become active earlier (*E*) and synchronously with region 2 (*F*, *G*). The early neurally evoked depolarization, however, is still evident. It does not appear when the cell is excited directly (*H*). The neurally evoked response arises at the skin end of the cell (near recording site 2) while the directly evoked response begins at the site of the applied stimulus (as in the inset). The zero potential is shown as the third trace. The resting potential of the cell is the deflection between traces 1 and 3. Calibrations: 100 mv. and msec.

of innervation by many nerve fibers are widely distributed over the surface of the excitable membrane. The electroplax of *Torpedo*, on the other hand, have lost the electrically excitable membrane as well, resembling in their properties only the end plates of muscle.

(8) Direct intracellular measurements on *Raia* electroplax have demonstrated that these are not electrically excitable.[44] *Raia* electroplax respond to stimulation of their nerves with a graded, long-lasting depolarizing potential which, at its maximum, reaches up to the level of zero membrane potential, or with perhaps only a few mv. of overshoot. Like those of *Torpedo*, the electroplax of *Raia* are homologues of muscle end plates.

(9) The long lasting PSP of cat[45] and toad[46] motoneurons are elicited only by neural stimulation of the cells and not by their antidromic or direct electrical excitation. These potentials are graded and can summate. They can develop during the refractory period of the cells following a spike.[4]

(10) The rabbit cervical sympathetic ganglion cell also responds with a PSP to neural stimuli.[47] This is longer than the spike, is graded and summating, and can be elicited by neural stimuli while the spike-generating membrane component is inactivated (FIGURE 6).

(11) The apical dendrites of the cat cortex develop a PSP.[48] When synaptic excitation is blocked by *d*-tubocurarine, the dendritic response cannot be evoked by direct or antidromic electrical stimulation[49] (FIGURE 7).

(12) Certain cardiac ganglion cells of the lobster respond to neural stimula-

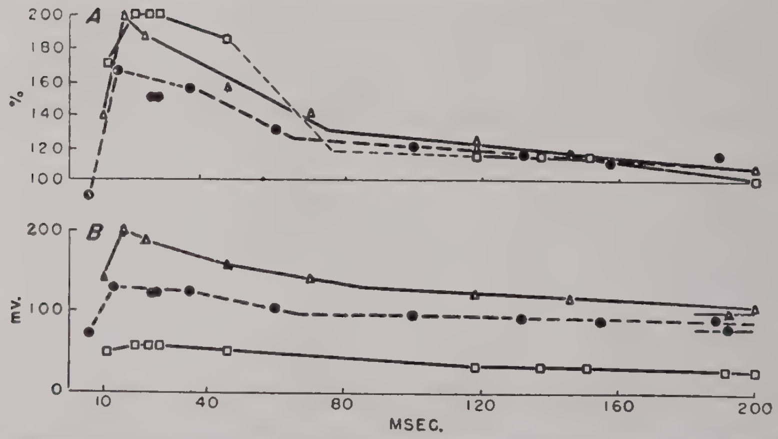

FIGURE 4. The time course of homosynaptic facilitation in eel electroplaques and the effect on this of eserine.[12] A strong conditioning neural volley excited the cells. A second, weak neural stimulus was delivered at various times thereafter. The height of the second response is shown (in mv.) as the filled circles in *B*. The amplitude of response in isolation (about 85 mv.) is seen as the asymptote at the end of the curve. The percentile change of the response is shown in *A*. Some of the cells were refractory at about 5 msec. interval between the stimuli, the test response being smaller than the control. A peak of facilitation, with the test response about 60 per cent higher than that of the control developed between 10 and 40 msec. after the conditioning stimulus, fell to 30 per cent at about 80 msec., but facilitation persisted more than 200 msec. Eserine (50 μg./ml.) was then added. Twelve minutes later the drug had enhanced excitability to the testing volley. This produced a larger response (about 100 mv.) seen as the asymptote to the upper curve of *B* (triangles). A larger amplitude of response was also produced on homosynaptic facilitation, but the time course of the facilitation was not significantly altered (*A*). The squares represent the facilitation 76 min. after continued eserine action, when the drug had depressed neural excitability of the cells. Expressed in percentile terms, the relative facilitation was larger than previously, but the time course of the effect was the same.

tion with depolarization, but the spike develops away from the soma of the cell, in the axon, and the spike does not invade the soma.[50] These cells therefore are also not electrically excitable.

(13) The giant neurons of *Aplysia* develop a PSP that is not elicited by direct stimuli,[51] and the PSP is not subject to refractoriness[52] (FIGURE 8).

(14) Both the hyperpolarizing (inhibitory) and depolarizing (excitatory) PSP can be reversed in an electrical sign by altering the electrochemical conditions of the cells in which the responses occur (FIGURES 9 and 10). This is not the case with the spike. The mechanism of the reversal is discussed below and is a consequence of the electrical inexcitability of postsynaptic membrane.*

Mode of Synaptic Electrogenesis

The membrane mechanisms that generate the depolarizing and hyperpolarizing PSP (FIGURE 1) are known only from indirect measurements.[27, 28, 53, 54]

* Marrazzi has raised the question "Is the adrenal medulla electrically excitable after denervation?" The data are conflicting but, as weighed by Rosenblueth,[106] they indicate that it is not. This neuron group belongs to the frankly neurosecretory class.[169] On the other hand, the majority of nerve cells, primarily exerting integrative activity, which demands conductile properties, are also secretory, but at restricted sites, the synaptic junctions. Nevertheless the circulatory distribution of their products is also an important control mechanism of the body (Purpura[7] and Rosenblueth[106]). Only the short-lived secretory products, such as acetylcholine, have a relatively confined zone of action. Since transmission at the adrenal medulla is cholinergic (Rosenblueth[106]), the synapse-activating drugs (acetylcholine and nicotine) cause secretion, while inactivators (*d*-tubocurarine, hexamethonium, and tetraethylammonium) block secretion normally caused by the stimulation of the splanchnic nerves.[161] The persistence of secretory responsiveness of the denervated gland,[161] however, suggests that a transmitter longer-lived than acetylcholine is involved in this excitation of the gland by circulatory agents. Histamine and serotonin may be implicated (von Euler[161]).

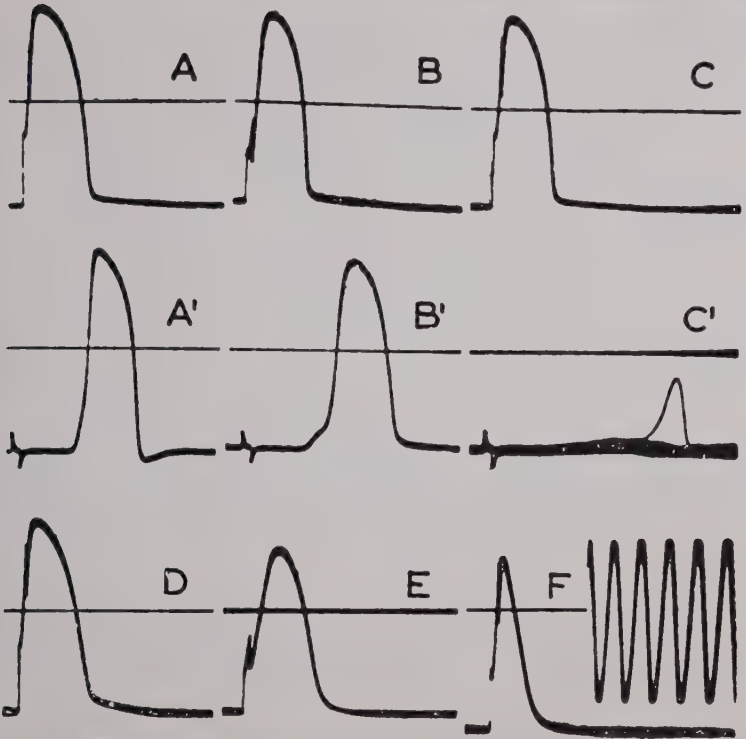

FIGURE 5. Effect of nondepolarizing synaptic blocking agents on the responses of the eel electroplaque.[12] Direct stimulation of the cell is represented in *A–F*, neural excitation in *A′–C′*. The initial responses to both stimuli are shown in *A* and *A′*. Three minutes after substituting a bathing solution containing 5 mg./ml. *d*-tubocurarine, the directly elicited spike was unchanged (*B*), but synaptic excitation was less effective, the spike arising later on the smaller PSP (*B′*). At 5 min. (*C, C′*) the directly elicited response was still unaffected, but the PSP had decreased so much (*C′*) that it was seen only with repetitive stimulation at 50/sec., and produced a single small "spike," after which a neural volley could no longer affect the electrically excitable membrane. The latter, however, remained fully responsive to a direct stimulus 41 min. later (*D*), but eventually this responsiveness decreased 96 min. later (*E*) and 110 min. after this, (*F*). The resting potential of the cell was unchanged. Calibrations 100 mv. and msec.

The depolarizing response is probably caused by increased membrane permeability to all ions and is associated[55] with a considerable decrease of membrane resistance.[27, 28, 31, 32] The hyperpolarizing PSP is probably associated* with permeability to either or both K^+ and Cl^-.[53, 54]

Since in all cases studied thus far the PSP of either type are not caused by electrical stimuli, their neural excitation must be mediated by other, probably chemical, agents. The nature of some of these will be discussed later. Some consequences of the inexcitability of postsynaptic membrane by electrical stimuli, however, further serve to characterize this electrogenesis and also to

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* Indirect evidence suggests that the PSP which are the responses of the *Torpedo's* electric organs are caused by an early rise of sodium conductance which develops an overshoot. On the other hand, the response of the electroplax of *Malapterurus* is probably a hyperpolarization.[5] The latter cells derive from glands, and both hyperpolarizing and depolarizing responses are found on neural or chemical stimulation of the salivary glands of the cat.

TABLE 1

COMPOUNDS THAT INACTIVATE THE POSTSYNATPIC MEMBRANE AND DO NOT DEPOLARIZE BUT CONVERT THE ALL-OR-NOTHING RESPONSE OF THE ELECTRICALLY EXCITABLE MEMBRANE TO THE GRADED RESPONSE

Substance	Minimum effective concentration µg./ml.
Eserine	25
D-Tubocurarine	50
DFP	100
Procaine	200
Tertiary analog of Prostigmine	1000
Flaxedil	—

explain its mode of behavior, which differs from that of the spike-generating membrane component.

Reversal of Sign of PSP

If the postsynaptic membrane that develops hyperpolarizing electrogenesis is itself hyperpolarized by an applied current, or if internal Cl^- is increased, the electrochemical gradient becomes reversed. The membrane, not being affected by these changes of the electrochemical potential, still responds to

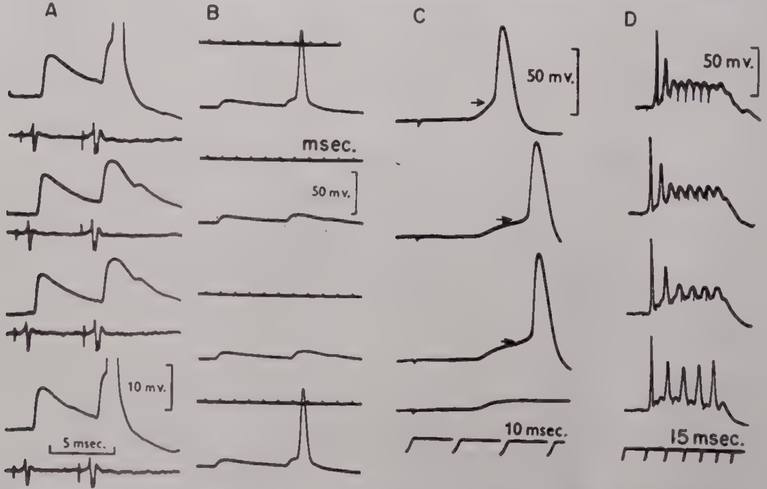

FIGURE 6. PSP and synaptic transfer in cat motoneurons (*A*, *B*)[45] and rabbit sympathetic ganglion cells (*C*, *D*).[17] A single afferent volley to the spinal cord produced a PSP too small to discharge the motoneuron, but the summation of PSP's at brief intervals between stimuli caused spikes (*1*, *4*). The summed depolarization at longer intervals (*2*, *3*) did not evoke spikes. Note that the presynaptic spike (lower line in each set of *A*) caused an insignificantly small depolarization of the motoneuron, too small to set up electrical excitation of the cell. PSP develops after a significant delay, which is also seen in other synaptic systems.[2, 5] Column *B* shows direct-coupled recordings at lower gain than was used in *A*. The PSP of the ganglion cell (*C*) is much longer than that of the motoneuron. Progressively stronger stimuli to the preganglionic nerve cause more rapidly rising depolarization and the spike is discharged earlier as the critical firing potential is reached earlier (arrows). The ganglion cell does not respond with full spikes to repetitive stimulation at 80/sec. At higher frequencies only one small second spike is produced, but the PSP are developed with each stimulus and maintain depolarization of the cell. The electrically excitable membrane that generates the spikes is inactivated by the depolarization.

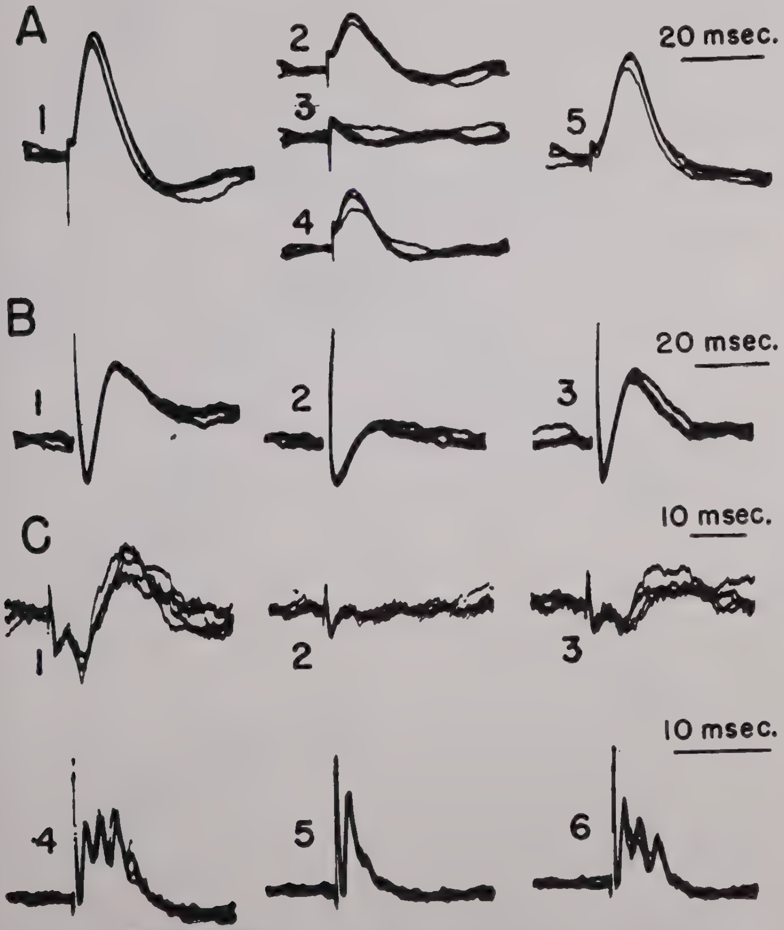

FIGURE 7. Electrical inexcitability of the postsynaptic membrane of apical dendrites of the cat cortex.[49] (*A*) Stimulating and recording from the cortex. The large normal surface negativity that is the response of cortical dendrites (1) is decreased 50 sec. after intravenous injection of 3 mg./kg. *d*-tubocurarine chloride (2). At 70 sec., it is gone (3), to return after 5 min. (4) and 20 min. (5). Four consecutive records superposed in each set. (*B*) Stimulating about 0.8 mm. below the cortical surface, recording as in *A*. The dendritic negativity (1) disappeared and only the positive response, representing the activity of the directly excited neuronal spikes, remained 45 sec. after an injection of the drug (2). Recovery was rapid, although not complete at 90 sec. (3). Three consecutive records superposed in each set. (*C*) The response of the motor cortex to stimulation of the pyramidal tract (1) is composed of two positive deflections preceding the negative dendritic potential. Two minutes after the injection of drug all synaptically induced activity (the second positive component and the negative dendritic response) disappeared, leaving only the first positivity which is ascribed to the antidromic response of pyramidal axons and/or cells in the deep cortical layers (2). Recovery after 20 min. (3). (4 to 6) From the same experiment, stimulating the cortex and recording in the pyramidal tract. The normal response is composed of a directly excited component and several synaptically evoked repetitions (4). The latter were absent 5 min. after the drug was administered (5) and recovered somewhat after 20 min. Five superposed responses in each set.

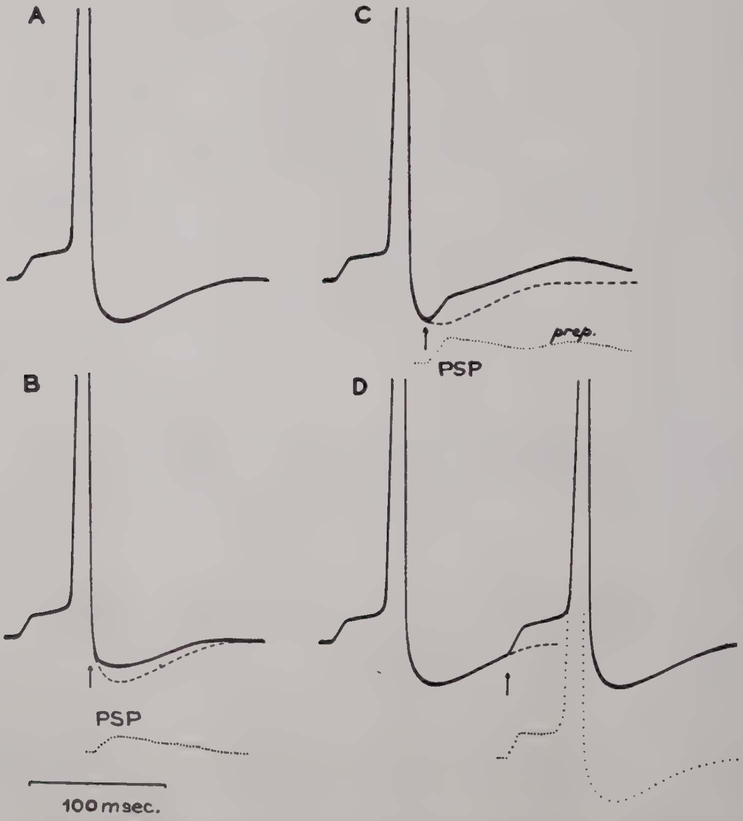

FIGURE 8. The responses of giant neuron of *Aplysia* to single and paired orthodromic stimuli. (*A*) A single shock evokes first a PSP out of which rises the spike. (*B*) The second stimulus, exciting the cell during its refractory period, adds a potential (solid line) to the initial response (broken line). The difference (dotted line, below) is due to the second PSP. (*C*) The second stimulus was delivered somewhat later. The added potential also shows a local response (prep) initiated by the PSP during the relatively refractory period of the electrically excitable membrane. (*D*) At a longer interval, a second stimulus evokes the full response, as in *A* (records kindly supplied by A. Arvanitaki-Chalazonites).

neural or chemical stimuli with the same conductance change as in the normal state. The ions involved, however, then flow in the opposite direction from the normal condition. Thus K^+ would flow inward, Cl^- outward, and the result would be a depolarization. This effect is found in cat motoneurons (FIGURE 9), in modified form in invertebrate muscle fibers,[57] and in crayfish stretch receptors.[58] The reverse obtains for the depolarizing PSP. A membrane potential more positive than the level of the equilibrium potential of depolarization produced by an applied current (FIGURE 10) or by the spike (FIGURE 11) reverses the sign of the response.* Direct evidence of reversal

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* This effect can also be obtained transiently with electrically excitable membrane,[59] but under circumstances that rapidly inactivate the membrane.[11] These secondary changes are apparently absent in the synaptically excitable membrane.

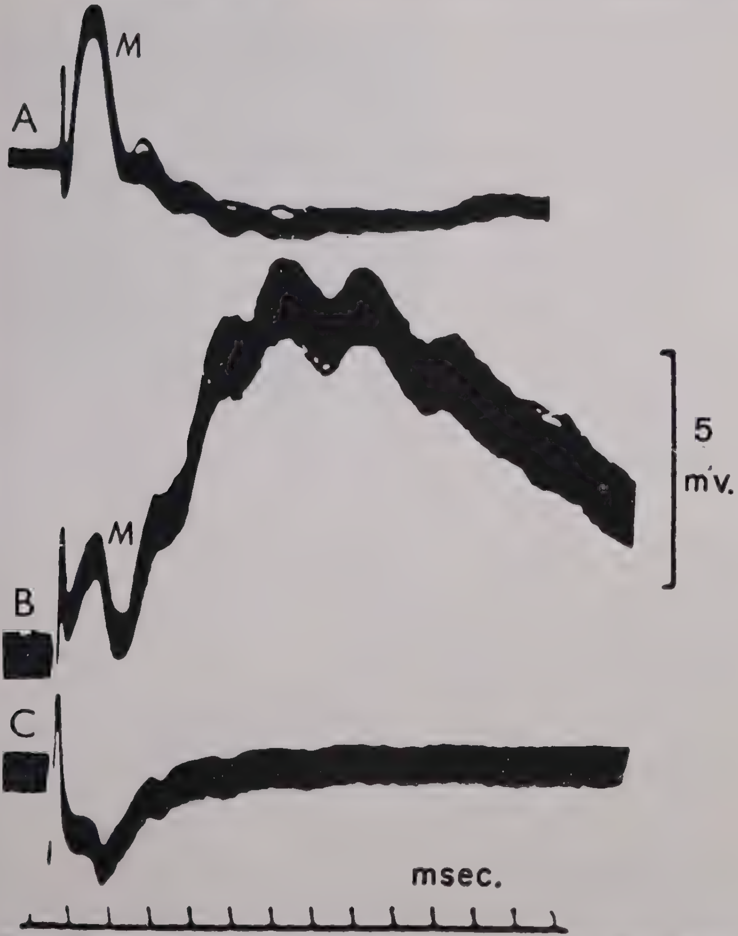

FIGURE 9. Reversal of an inhibitory, hyperpolarizing PSP into a depolarizing, cat motoneuron.[53] The initial deflection *M* is the antidromic spike that did not reach the soma. Following it (*A*) is a hyperpolarization generated through activation of inhibitory postsynaptic membrane by Renshaw cells excited through the motoneuron collaterals. Passing current through the recording electrode then increased the internal Cl. Activation of the inhibitory postsynaptic membrane now led to a depolarizing potential indicating outflow of Cl. (*C*) Extracellular responses on withdrawing the microelectrode from the cell show the reversed *M* spike, but not the PSP.

of the depolarizing PSP has been obtained with intracellular stimulation of, and recording from, frog "slow" muscle fibers in which only a PSP occurs.[34]

Graded Responsiveness and Summation of Electrogenic Activity in Postsynaptic Membrane

The graded summative nature of the PSP is another consequence of electrical inexcitability. The potential resulting from neural or chemical excitation

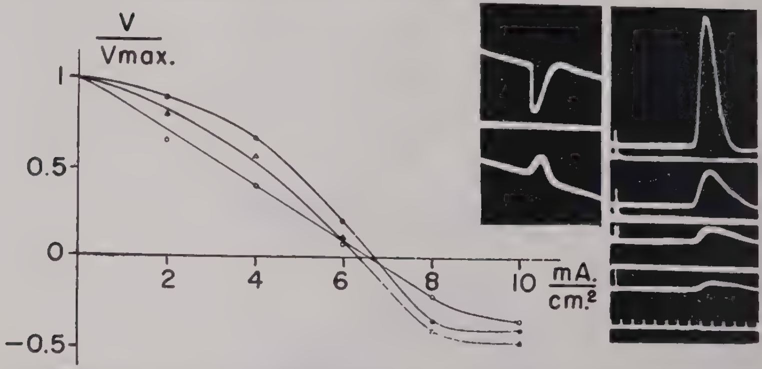

FIGURE 10. Changes of the response of *Torpedo* electric organ subjected to various strengths of depolarizing current.[5] Right: the portion of the organ produced about 12 v. on discharge (upper record). The response decreased with progressively increasing depolarizing currents. Left: the upper record is the response without a depolarizing current (recording connection reverse of the previous set). An extremely strong current inverted the response. The graph shows 3 similar sets of experiments, the applied current on the abscissa and the ordinates representing the discharge voltage as a fraction of the initial response. In all cases the sign of the discharge was inverted with strong applied currents This behavior does not correspond with that of a spike, but can be ascribed to a PSP

does not in turn affect the responsive membrane, the latter developing its electrogenesis only in proportion to the amount of the initial specific stimulation. For the same reason the postsynaptic membrane is not inactivated by strong or prolonged depolarization as is the spike (FIGURE 12), and is therefore not subject to refractoriness. Both factors operate to permit long sustained summation of the PSP, as shown in FIGURE 6.

MODE OF TRANSFER BETWEEN PSP AND SPIKE

Excitation

When a depolarizing PSP develops in membrane that also has an electrically excitable component, the synaptically evoked electrogenesis is capable of acting as a stimulus to this component. Weak excitatory PSP evoke only local responses confined to the electrically excitable membrane in the immediate region of the synaptically responsive membrane (FIGURES 8, 12). When the excitatory PSP reaches a larger magnitude, its depolarization and that of superimposed local responses summate to evoke a spike (FIGURE 3).

Inhibition

By hyperpolarization. On the other hand, hyperpolarizing PSP are inhibitory and can modify or suppress the development of spikes by excitatory stimuli (FIGURE 13). Once a spike is elicited it is difficult to suppress it by hyperpolarizing stimuli.[2, 60] The algebraic summation of depolarizing and hyperpolarizing PSP, however, can make for critical differences in the net excitatory value of synaptic inflows and, thereby, inhibitory PSP can modify the over-all responses importantly and dramatically.

By depolarization. Inhibition can also be developed by overexcitation through

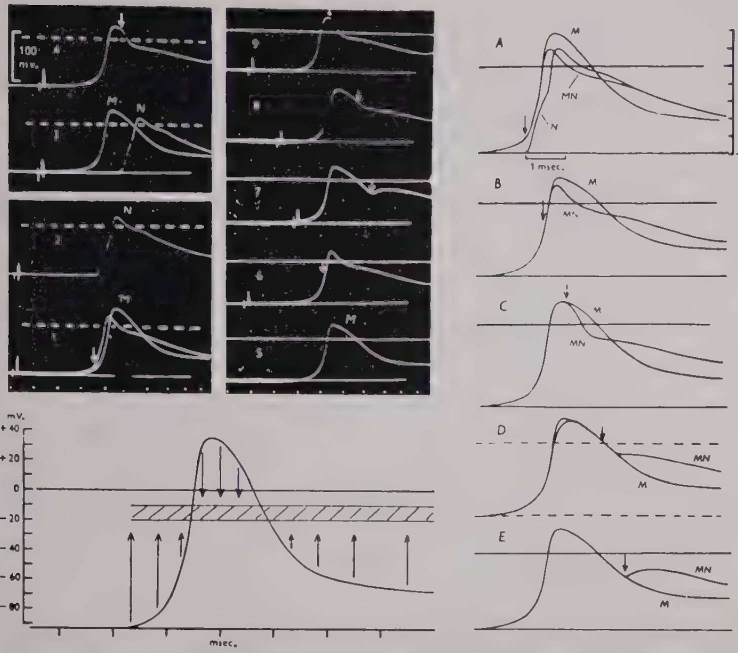

FIGURE 11. The distortion of the spike of frog muscle by the end plate potential.[28] Lower left: when recording the directly excited spike in the region of the end plate, a superimposed EPP elicited by stimulating the motor nerve at first adds to the depolarization caused by the spike; in the region of overshoot it decreases the spike; as the spike returns to internal negativity, a superimposed EPP again raises its potential. This reversal takes place in a range of −10 to −20 mv. that represents the level of the diffusion potential for the completely depolarized postsynaptic membrane. Upper left: experimental records, *M* being the directly excited and *N* the neurally excited responses. In records 4, 6–9, a nerve volley superimposed on the direct spike at different times, shown by arrows. Right: analysis of the records showing the distortion of the *M* spike produced by a superposed neural volley, *M N* spike (composed from several figures of the reference).

the depolarizing postsynaptic membrane component. This effect is exerted by the long-term inactivation process or processes to which the electrically excitable membrane is subjected by depolarization. It is seen on chemical or electrical depolarization of the electrically excitable membrane itself (FIGURE 14), or through the prolonged operation of depolarizing postsynaptic electrogenesis (FIGURES 6, 12).*

Transfer in Muscle Fibers

The processes that develop the spike of "twitch" muscle fibers also initiate contractions through an as-yet-unknown transfer mechanism. The inactivation of the spike, as, for example, by prolonged depolarization of the cell, often also leads to disappearance of the contractile response. The degree of specific coupling between the spike and contractility, however, apparently differs in various muscles. In some, depolarization is itself a sufficient stimulus for the

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* This mechanism accounts for classical Wedensky inhibition.

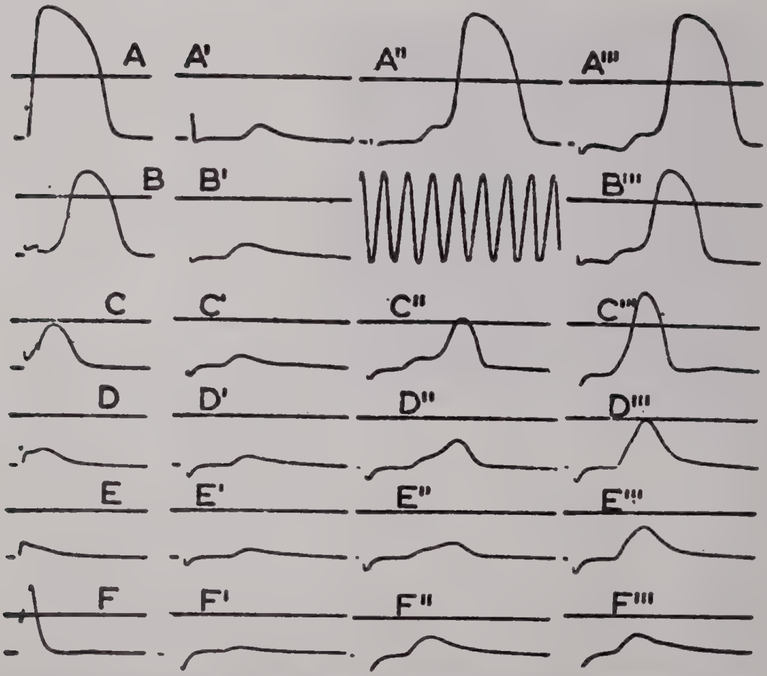

FIGURE 12. Differential effects of depolarization on the spikes and PSP of the eel electroplaque.[12] Column *A–F*, direct stimulation; columns *A′–F′*, etc., weak, moderately strong, and very strong stimuli to the nerve. *A–A′′′*, the response of the normal cell. The strong direct stimulus evoked a spike with very brief latency (*A*). The weak neural volley caused a PSP (*A′*), the stronger also a spike (*A′′*, *A′′′*). The cell was then treated with weak eserine (25 μg./ml. solution) for 78 min. and weak acetylcholine (1 μg./ml.) for the last 58 min. of that period. These drugs had no effect on the potentials. Five μg./ml. acetylcholine were then added. Depolarization developed, the spikes 36 minutes later becoming smaller, but the PSP was unaffected (*B-B′′′*). The diminishing electrically evoked response 9 min. later (*C–C′′′*) became graded, as seen by its larger size in response to the strong neural volley. These effects progressed during the next 19 min. (*D–D′′′*) and 11 min. thereafter (*E E′′′*). The PSP to the threshold neural volley decreased (*E′*), but was still evident (*F′*) when the electrically excitable membrane no longer responded to a much stronger direct stimulus (*F*). The PSP to a maximal neural stimulation (*F′′′*) was still about as large as initially (*A′′′*). This PSP was still capable of evoking a small graded response of the electrically excitable membrane, as seen by the small delayed additional potential on the falling phase. Calibrations 100 mv. and msec.

contractile system. When these fibers are depolarized by chemical or electrical means, sustained contractions or "contractures" are observed. The contractile system of the frog "slow" muscle fibers also responds to graded membrane depolarization, which is a PSP and is the only mode of electrogenesis of which these fibers are capable.[33, 34] The graded, summative, and prolonged potentials that are their characteristic electrical responses lead to graded, sustained "tonic" contractions of these muscle fibers.[2] While precise electrophysiological data on most kinds of smooth muscle are lacking, nevertheless it is possible to explain actions of various drugs[61] on the basis of similar transfer between graded, chemically excitable electrogenesis and graded "tonic" contractions (see below, under *The Pharmacology of Specific Peripheral Synaptic Systems*).

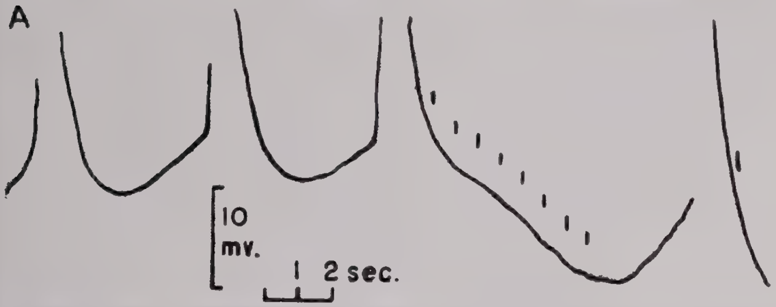

FIGURE 13. Effect of vagus stimulation on the membrane potential of frog sinus venosus. Rhythmic depolarizations of the cell grow into discharges of spikes. A series of stimuli to the vagus nerve (shown by the dashes) cause increased polarization of the membrane which suppresses the discharge.[93]

Repetitive Responses

The rate of inactivation of the electrically excitable membrane appears to differ considerably in different tissues, and it is probably responsible[5] for the specifically different rates of "adaptation" of various cells (FIGURE 15). Different kinetics of inactivation, specific critical firing levels for the spike (FIGURES 3, 6), and specific time constants of the electrically excitable membrane[62] can account for different patterns of repetitive firing of various types of neurons, as observed in many cells.[5, 51, 57, 63, 64]

SYNAPTIC DRIVES AND NEURONAL COMPLEXITIES AS FACTORS IN EXCITATION AND INHIBITION

A given neural stimulus evokes differently effective PSP at different loci of a cell (FIGURE 3). The reasons for these spatial differences in synaptic drive are not as yet known, but they may derive from different amounts of transmitter released by various presynaptic endings; from variations in the density and extent of these endings; from the specific sensitivities to the transmitter of the different postsynaptic loci; and from the spatial orientation of postsynaptic and electrically excitable regions. The same factors probably determine also the different effectiveness of one or another neural pathway for the same final effector cell (FIGURE 16).

Complexity of synaptic pathways, in terms of synaptic drives, in the number of interneuronal chains (FIGURE 17), and in their specific excitatory and inhibitory actions have been extensively studied in the relatively simple synaptic systems of the spinal cord[65, 66, 67] and in the invertebrate nerve cords.[68, 69] Even greater complexity probably exists in the higher levels of the vertebrate central nervous system, and an analysis of some of this complexity is presented later in this paper and, by Purpura, elsewhere in this volume.

Actions of Drugs on Peripheral Synapses

GENERAL CONSIDERATIONS

The special vulnerability of junctions to drugs has been recognized since Claude Bernard's discovery of the curare effect on neuromuscular transmission.

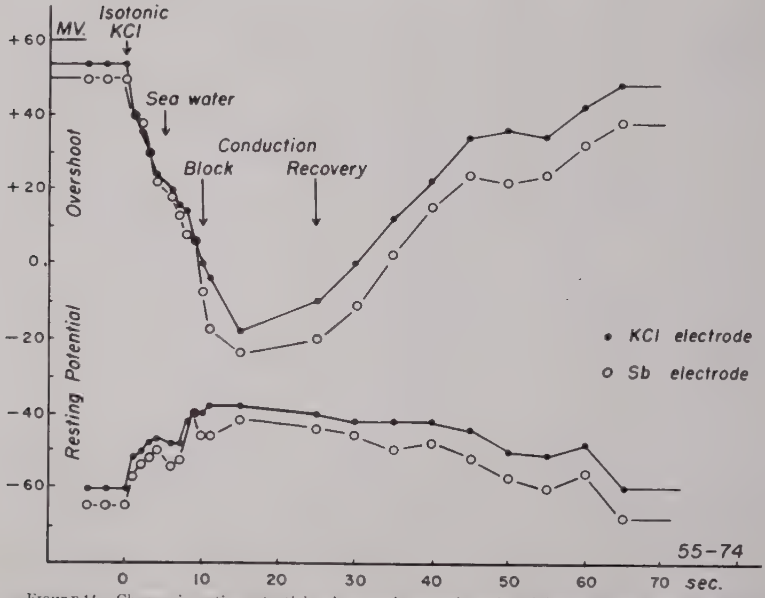

FIGURE 14. Changes in resting potential and responsiveness of a region of the squid giant axon. Two micro electrodes, one a 3 M KCl-filled microcapillary and the other a glass-insulated antimony wire, were inserted within 1 mm. of each other in the center of a 1.2 cm. length of the axon separated by vaseline seals from the rest of the fiber. The resting potentials and spikes recorded with the two electrodes were nearly identical. Isotonic KCl was then placed in the central well, causing depolarization and decrease in the spike. The depolarization continued while the KCl was being washed out with sea water, the response blocking, as seen by recording from the distal segment of the axon. Recovery of responsiveness set in while the depolarization of the initially blocked region was still almost as large as when block had developed. Spike amplitude increased much more rapidly than did the resting potential (unpublished data, C. Y. Kao and Grundfest).

Subsequent work emphasized the junctional effects, particularly in terms of the autonomic nervous system.[70] An important development, derived from work with smooth muscle, was the concept of "receptor sites" reacting to epinephrine with "excitatory" (motor) responses or with "inhibitory" (relaxation).[71] Nevertheless, probably because of the anatomical complexities and lacunae in the knowledge of smooth muscle, no correlation was made between receptor sites and electrogenic effects. Also, the relatively large variety of excitatory and inhibitory receptor sites and a large variety of interactions in the effects of drugs brought forth a complex nomenclature to describe drug actions.

A linkage of drug actions and electrogenic synaptic activity began to be sought[72] with progressively more precise techniques soon after the demonstration of synaptic potentials in muscle,[73] and in the autonomic[74] and central nervous systems.[45] The data, especially clear in the case of muscle synapses,* showed that depression and abolition of neuromuscular transmission by curare was associated with a decrease and eventual block of EPP. Other compounds, especially those linked with acetylcholine, the generally accepted transmitter of cholinergic systems, were found to elicit EPP and spikes,[76] or to enhance the EPP and its excitatory effects. Similar results were also obtained on sympathetic ganglia.[76a]

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* The review by Hunt and Kuffler[75] contains a more detailed correlation of electrogenesis and pharmacology in neuromuscular transmission than was possible in this paper.

An important advance in relating pharmacological action and electrogenesis

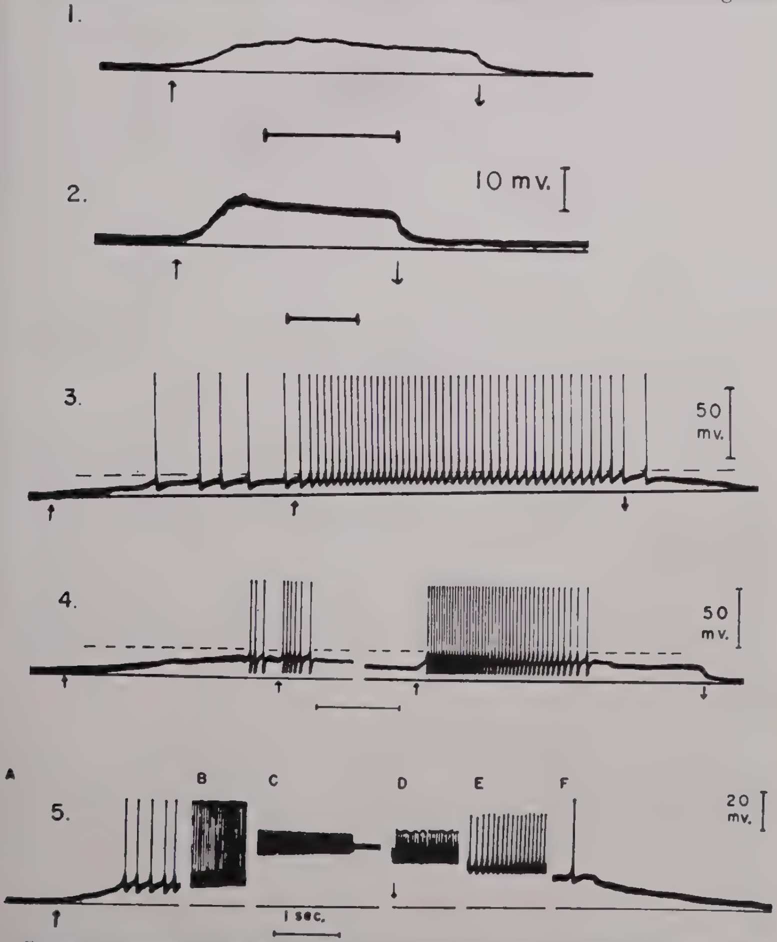

FIGURE 15. Excitation, adaptation, and inactivation in a sensory cell, the crayfish stretch receptor.[86] The potentials recorded with an electrode in the soma of the cell were produced by stretching the terminal sensory dendrites that have mechanotransducer membrane. In all records beginning of stretch, or an increase is indicated by the upward arrows; release of the tension by downward arrows. (*1* and *2*) Records at high amplification on weak stretch to a slowly adapting receptor (*1*) and a rapidly adapting receptor (*2*). The constant stretch stimulus did not maintain a steady depolarization of the cell, the potential declining gradually. (*3*) Progressively increasing stimulation of a slowly adapting receptor. A weak stimulus (but stronger than that in *1*) evoked a series of spikes discharging at a low rate. Increasing the stretch caused a rapid burst and then a fairly steady discharge until the stimulus ended. A single "after discharge" occurred while the depolarization was still large. (*4*) Discharge is not maintained in a rapidly adapting cell. As the level of depolarization caused by a steady stretch drops, the discharge stops, but is started again at a higher frequency when the stretch is increased. (*5*) Increasing the stretch stimulus to a slowly adapting cell raises the level of depolarization and blocks the spikes (*C*). On gradually decreasing the stimulus, recovery from this inactivation of the electrically excitable membrane is slow (composed from several figures of the reference).

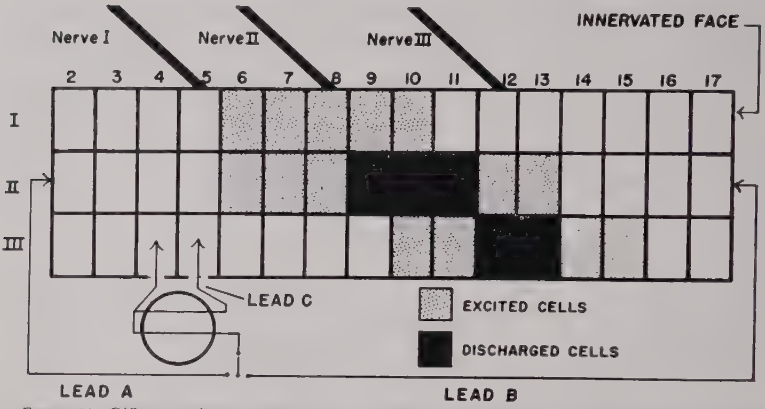

FIGURE 16. Different excitatory effects of 3 nerve trunks innervating the same electroplax of the Sachs Organ.[43] One row of 16 cells is shown successively in I, II, and III. Excitation of nerve trunk I did not discharge any of these electroplaques, but facilitated the subsequent excitation of cells 6 to 10 by a second homosynaptic volley. Nerve II innervated cells 6 to 13, a single volley discharging only cells 9 to 11, but facilitating the excitability of all 8 cells to a second volley. Nerve trunk III excited cells 10 to 15, discharging cells 12 and 13 of these. External leads *A*, *B*, and *C* were the means used to identify these effects.

was made by Paton and his associates,[77, 78] who concluded that blocking agents such as *d*-tubocurarine effected this action without depolarizing the postjunctional cell, while acetylcholine itself and the group of transmitter analogs depolarized and excited muscle fibers or autonomic ganglion cells before blocking transmission. This classification is modified in its concept as used below. The "depolarizing" drugs activate synaptic electrogenesis, whereas the "nondepolarizing, competitive, antagonistic" agents inactivate this. Certain complications, particularly evident in the case of "depolarizing" drugs,[78-81] can be taken into account as probably due to the complexities of postsynaptic membrane and to its undoubted lability in reaction with chemical agents (see also Grundfest[5]).

THE PHARMACOLOGY OF SPECIFIC PERIPHERAL SYNAPTIC SYSTEMS

In the following, the action of drugs on progressively more complex peripheral synaptic systems will be analyzed in terms of their effects on the electrogenic mechanisms. Certain generalizations will then summarize these data and will be applied to describe the pharmacology of some central nervous synapses.

Cells that Generate Only the Depolarizing PSP

(1) *Electroplax.* The most clear-cut of these systems is that of *Raia* electroplax which respond only to neural stimuli and with graded, depolarizing PSP. The preliminary account of this work,[44] however, does not provide pharmacological data.

Torpedo electroplax have long been an object of study, but much crucial electrophysiological information is nevertheless still lacking. The weight of evidence[5] indicates that these cells respond only with a depolarizing PSP and

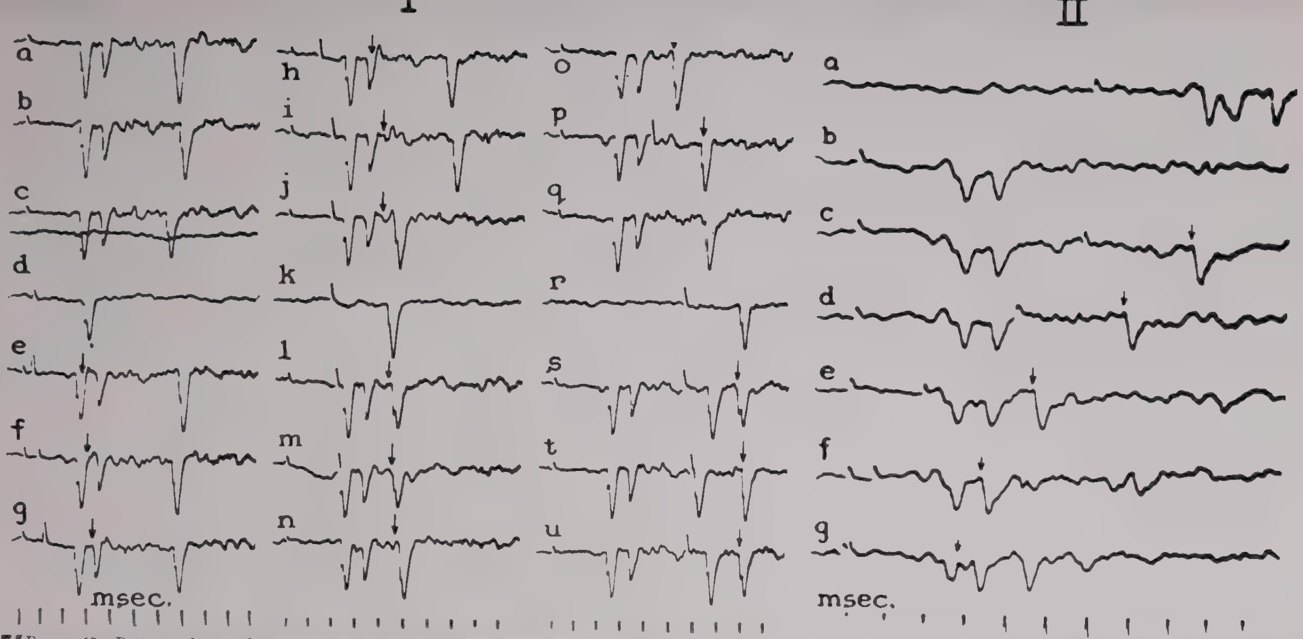

FIGURE 17. Patterns of synaptic organization and effects in the cat spinal cord.[65] Two different afferent pathways excited the cells of Clarke's Column, and each evoked a different, but characteristic pattern of activity recorded from a few fibers in the dorsolateral funiculus. (*I*, *a* to *c*, *q*) Stimulating the peroneal nerve caused repetitive discharges in two fibers, the first repetition at 1000/sec., the second after a delay of about 3 msec. The constancy in the pattern of the response is illustrated in the 4 records, taken over an interval of about 1 hour. (*d*, *k*, *r*) A hamstring volley, on the other hand, evoked only a single discharge in the two fibers. The other records of the first 3 columns show the effect of the hamstring volley at different intervals after the peroneal. Arrows show when the single discharge to the hamstring volley should have developed. Absence of the volley in records *e* to *j* or *p* proves that the responses to the different volleys developed in the same final common paths. A late response evoked by the interaction of the 2 pathways is seen in *j*. When this or the discharge to the hamstring volley itself occurred (*e* to *n*) the interposed response inhibited the later discharge of the peroneal volley. Facilitation (*j*) and inhibition (*l* to *n*) therefore are disclosed in the interneuronal complex that caused the repetitive discharge to the peroneal volley. (*II*) Another experiment. A tibial volley caused a triple discharge (*a*), while the peroneal developed 2 (*b*). The latter inhibited the late discharges to a tibial volley which followed 6 msec. later (*c*). When the two stimuli were closely spaced (*g*), the tibial volley caused no direct discharge (arrow shows the time at which the missing response should have occurred). A late discharge was evoked, however, indicating that at the brief interval excitatory effects predominate over the inhibitory at the interneurons.

only to neural stimuli. Curarization, like denervation, renders the cell unresponsive.[39] The close injection of acetylcholine causes prolonged, graded depolarizing electrogenesis.[80] *Torpedo* and *Narcine* electroplax apparently belong in the cholinergic group.[82, 83] The pharmacological responses of *Torpedo* thus accord with this classification.*

(2) *Salivary gland cells.* Some cat submaxillary gland cells respond with depolarizing graded electrogenesis to stimulation of their sympathetic and parasympathetic innervations.[56] The electrogenesis is presumably associated with secretory activity. The pharmacological responses of these cells are not known, but other cells that respond only with hyperpolarization to both types of neural stimuli also yield hyperpolarization with acetylcholine, epinephrine, and pilocarpine.

(3) *"Slow" muscle fibers.* The pharmacology of these "tonically" contracting fibers in the frog, as yet not known, should provide very important information particularly useful to the interpretation of the pharmacology of other muscles, including the smooth. The electrogenesis of these cells is entirely a graded, sustained depolarizing PSP[34] that causes similarly graded and sustained contraction.[33]

Cells That Produce Only a Hyperpolarizing PSP

Electrogenesis solely of this type probably would serve no useful function in neurons or muscle fibers. It occurs in gland cells,[56] however, and, in combination with depolarization caused by specific stimuli, in a sensory cell,[58] being capable of usefulness in both kinds of cells.

Salivary gland cells of this type have been mentioned in (2) above, and they should provide important data for pharmacology. For example, these cells could yield information as to whether the hyperpolarizing responses are generated in a single membrane component responsive to both adrenomimetic and cholinomimetic drugs, or whether the same cell is endowed with differently excitable, but similarly electrogenic, membrane components. This question is probably also of importance for various types of smooth muscle.

Hyperpolarizing electrogenesis is also found in the electric organs of *Malapterurus*, whose cells are derived from glands of the skin. Unfortunately, during the past half-century,[5] almost no electrophysiological nor pharmacological studies have been made on this fascinating and potentially important material.

The hyperpolarizing PSP produced at the dendrites of crayfish stretch receptors[58] diminishes the depolarization that develops in the soma when the terminal dendrites of the cell are stretched.[86, 87] This action decreases the frequency of, or inhibits impulses generated in, the electrically excitable membrane of the cell. While the mechanoreceptor membrane of the terminal dendrites is also activated by acetylcholine,[58] the pharmacology of the inhibitory synapses is not yet known.

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* Chagas[84] states that he has been unable to block responsiveness of *Torpedo* electroplax with Flaxedil. No details are given, and the conflict with earlier work using curare[85] awaits resolution.

Cells with a Depolarizing PSP and a Spike

Eel electroplax. The pharmacology of these cells is analyzed in some detail because of the availability of a considerable body of such data,[12, 84] as well as of a rather thorough knowledge of the electrogenesis.[2]

The single innervated cell generates only a PSP in response to a weak nerve volley (FIGURE 3). The response occurs simultaneously at many sites of the excitable membrane, indicating the diffuseness of the innervation by a number of fibers from the same nerve trunk. Several trunks supply fibers to each cell (FIGURE 16). The PSP is almost always largest at one region of the cells (FIGURE 3) and, with increasing stimulation of the nerve, it grows more rapidly at this site than it does elsewhere. The PSP initiates a local response of the electrically excitable membrane and the two depolarizing potentials sum. When this depolarization reaches about 30 to 40 mv. a spike is initiated that propagates away from the most excitable site. Not all of the nerve trunks supplying a given cell cause a sufficiently large PSP to produce a spike (FIGURE 16), the cell then remaining highly excitable to a subsequent stimulus for about 1 second (FIGURE 4). In many cells the spike is elicited by a submaximal neural volley, and stronger stimuli to the nerve then cause large enough PSP at other sites of the excitable membrane to produce nearly simultaneous development of the spike everywhere in the cell (FIGURE 3). Therefore, although the synaptic potency of different regions of innervation is different, the capacity of the PSP to excite the spike-generating membrane is everywhere similar. With respect to multiplicity of innervation of different potencies and of the diffuseness of the junctions, the eel electroplaque resembles a neuron more closely than it does the muscle fiber. Like skeletal muscle, however, this system lacks inhibitory PSP. The nature of the transmitter agent of eel electroplax is not yet determined. In its reactions to various drugs this synaptic system appears to be cholinergic, like that of skeletal muscle. Injection of acetylcholine, however, does not excite the electric organ to discharge, nor do the anticholinesterases change the form of the PSP or the time course of facilitation.[12] These discrepancies may be of only minor significance.

The first effect of the nondepolarizing compounds (TABLE 1) is to diminish and eliminate the PSP (FIGURE 5). Neural excitation of the cells is then blocked, but they remain responsive to direct stimuli. Alteration of the electrically excitable membrane also takes place, eventually inactivating the latter.[12] This matter will be discussed later under the heading *Competitive Antagonistic Actions.*

TABLE 2

COMPOUNDS THAT DEPOLARIZE, INACTIVATING THE ELECTRICALLY EXCITABLE MEMBRANE, BUT NOT THE POSTSYNAPTIC MEMBRANE

Acetylcholine	5
Carbamylcholine	10
Decamethonium	10
Dimethylaminoethyl acetate (DMEA)	50
Prostigmine	50
Succinylcholine	—

The substances that have been found to exert these effects also exert the same type of action on muscle and autonomic ganglia.[77, 78] The variety of the synaptic blocking agents studied with the electroplax preparation is as yet limited, but it ranges from those classically recognized as such (*d*-tubocurarine and Flaxedil), through anticholinesterases (eserine, DFP, and a tertiary Prostigmine) to an anesthetic (procaine). The concentrations required vary considerably, probably indicating that the postsynaptic membrane components combine more readily with some drugs, and less readily with others. Nevertheless, the primary effect is the same in all cases, inactivation of postsynaptic electrogenesis without depolarization of the cell,* and it shows a slower action upon the electrically excitable membrane.

The "depolarizing" drugs, also ranging widely in structure and function, and probably not at all limited to the group thus far studied (TABLE 2), likewise have a common type of action that differs fundamentally from that exerted by the nondepolarizing group. The cell undergoes depolarization (FIGURE 12), which results first in a decrease of the spike amplitude, then in a loss of all-or-nothing responsiveness and, eventually, in block of the electrically excitable membrane. This effect, similar to that produced by depolarization of axons or muscle fibers by KCl (FIGURE 14), is ascribable† to "sodium inactivation."[11] The PSP, however, can be produced after the spike-generating membrane is inactivated.

The effects evoked by the depolarizing drugs appear to be related to their action upon the postsynaptic membrane, these compounds exciting the latter to produce its sustained electrogenesis. Addition of the transmitter through neural excitation develops a PSP superimposed on the depolarization. Thus, the "depolarizing" drugs act in a manner similar to that of the transmitter but, being present in quantity in the medium, they cause a sustained depolarization rather than the temporary response of the PSP. Probably because the chemically sensitive postsynaptic membrane is widely disseminated throughout the excitable surface of the eel electroplaque, the depolarization is everywhere identical and can assume a large value, eventually approaching complete depolarization of the cell.‡ The magnitude and rate of depolarization are functions of the amount of drug used and of its time of action.

Skeletal muscle. The electrophysiological data on the synaptic mechanisms of muscle fibers are more complete than on any other system, due to their long study by many workers, and chiefly from the recent work of Katz and his colleagues (for a summary see del Castillo and Katz[32]). The synaptic apparatus is concentrated at one or at a few anatomically distinct restricted regions of the muscle fiber. Each end plate is probably supplied with a single nerve fiber, and it generates an EPP, the duration of which is long in comparison with that of the spike, unlike the case in eel electroplax.

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* A secondary depolarization occurs with DFP, which will be discussed later.

† It is probable that complete depolarization of the cell eliminates also the PSP, since that electrogenesis is linked to the electrochemical potential as described above.

‡ The term "depolarization" as used in this paper and, generally, in current literature is not precise. The word, as originally and correctly used by Bernstein,[15a] means complete disappearance of the membrane potential. In current usage it is employed to describe any change from the resting potential, from a minimal value to complete depolarization. Whenever it is more convenient, the term "depolarizing" is therefore used in the present discussion. It is probable that elementary electrogenic units do undergo complete electrical shifts from one level of potential to another during their response. The observed effect, however, is an average potential change, its magnitude depending upon the relative proportion of electrogenically active and inactive membrane.

At the neuromuscular junction, transmission appears at its simplest and most straightforward. Probably in most, if not all, cases, a single nerve impulse normally generates enough transmitter agent to evoke an EPP sufficiently large to excite the spike-generating membrane immediately in the vicinity of the postsynaptic.

Transmission can be blocked if the nerve does not release enough excitant to evoke a large EPP, if the responsiveness of the postsynaptic membrane is decreased, or if the excitability of the spike-generating membrane is depressed. All these cases are known under conditions of physiological or pharmacological experiments. An important aspect of the localization of the end plates, which enters into later consideration, is that the depolarizing PSP spreads passively by electrotonus and is largest in the immediate vicinity of the end plates, rapidly decaying with distance away from the membrane sites.[27]

d-Tubocurarine, a nondepolarizing drug, depresses the magnitude of the EPP and thereby impedes and eventually blocks transmission, as the EPP falls below the critical level required to excite the spike. The electrically excitable membrane, however, is not affected,[*] and the fiber remains responsive to direct stimulation. Other drugs of this category act likewise[77] (TABLE 3).

Acetylcholine and other depolarizing drugs at first produce contractions of the muscle fibers.[75] These undoubtedly result from the stimulation of the end plates by the chemicals and the production of EPP which, however, is sustained as depolarization by the continued presence of the drugs.[†] Sustained depolarization, however, inactivates the electrically excitable membrane of most muscle fibers. Therefore, following an excitatory phase, the depolarizing drugs produce block of excitation of the spike, although it is probable that the EPP is still elicited by neural stimuli. Certainly, the postsynaptic membrane still remains reactive to acetylcholine when the cell is fully depolarized.[31]

On the other hand, a spike is probably not necessary for contraction in some types of skeletal muscle (as, for example, in the slow fibers of frog). Depolarization by the action of the synaptic drugs should lead to sustained contractions or contractures in such muscle. This type of response is common in muscles of the frog and of birds, and in denervated muscle of the cat.[90]

The degree of inactivation of the electrically excitable membrane may differ in different types of muscle, depending on the amount of depolarization produced at the end plate region (or regions, if there are several) and the electric circuit properties of the muscle fiber. One may therefore expect that some types of muscle fibers will remain electrically excitable to some degree, while others will be totally inactivated. Skeletal muscle fibers treated with decamethonium thus remain electrically excitable when stimulated at their nerve-

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[*] It is not known why the direct effects on the electrically excitable membrane seen in the eel electroplax are absent in muscle fibers or axons. One possibility is that the former, with its much lower membrane resistance,[89] is penetrated by the drug while the latter is not. Injection of *d*-tubocurarine into the squid giant axon depolarizes the latter as well as causing block of the spike.[13a, 13b] The drug applied externally in very high concentrations does not affect the axon. On the other hand, the similarly acting inactivating drug, eserine, does affect muscle and axon when externally applied, changing the all-or-nothing response into graded, and then blocking, excitability.

[†] These muscle responses, usually termed "fasciculations," are in reality due to direct excitation of the individual fibers by the depolarization at the end plate regions. Therefore they should be called "fibrillations *en masse*" since many fibers are simultaneously active, respond repetitively, and therefore produce massive contractions, but do not involve the entities of motor units.[6]

TABLE 3

EFFECTS IN 3 SYNAPTIC SYSTEMS OF DRUGS ACTIVATING SYNAPTIC ELECTROGENESIS (PART A) OR BLOCKING IT (PART B)*

	Eel electroplaque	Muscle end plate	Ganglion
Part A			
Acetylcholine	+	+	+
Succinylcholine	+ (*P*)	+	?
Carbamylcholine	+	?	?
Decamethonium	+	+	−
Tetramethylammonium (TMA)	?	+	+
Nicotine	?	+	+
Prostigmine	+	+	+
TMEA	+	?	?
Part B			
D-Tubocurarine	−	−	−
Gallamine	− (*P*)	−	?
Pentamethonium	?	−	−
Hexamethonium	?	−	−
Tetraethylammonium (TEA)	?	−	−
Eserine	−	?	−
Tertiary Prostigmine	−	?	?
DFP	−	?	?
Procaine	−	?	?

* This table is chiefly a combination of TABLES 1 and 2 and of the summary by Paton and Perry.[78]

Symbols: + = activation, in these systems manifesting itself as depolarization; − = inactivation, synaptic block without depolarization; ? = data not available; *P* = collateral data indicate probability of the designated effect.

free regions. The spike propagates toward the end plate zones, but stops there.[77]

Autonomic ganglion cells. Earlier electrophysiological data have been recently confirmed[47] with intracellular recordings from single orthodromically stimulated cells of the cervical sympathetic ganglion of the rabbit. Nerve stimulation produces graded, summating depolarizing PSP which, in turn, evokes a spike. Repetitive stimulation at high rates elicits only a few spikes (FIGURE 6), the later ones being depressed in amplitude. Persisting throughout the period of stimulation is a large depolarization caused by the summating PSP. The absence of spikes is probably due to rapid inactivation of the electrically excitable membrane by the depolarizing electrogenesis.[5] Depression of synaptic transmission by the nondepolarizing drugs is well known[78] (TABLE 3).

The depolarizing drugs first cause excitation denoted by impulses propagating in the postganglionic fibers.[76] The depolarization then blocks transmission as sustained preganglionic excitation does (FIGURE 6) and as the drugs act in the eel electroplax and muscle fibers. Decamethonium, however, does not depolarize the cat's superior cortical ganglion but, in high concentration, it acts as *d*-tubocurarine does.[78] Thus, this postsynaptic membrane, although responding in general to the same types of drugs and in the same way as do the homologous components in muscle and the eel electroplax, appears to have some specific differences. TABLE 3 compares the pharmacological reactions of the 3 types of postsynaptic membrane.

Cells with Both Depolarizing and Hyperpolarizing PSP

The only known case which falls into this category is that of some salivary gland cells.[56] In these cells the different electrogenic modes—depolarization on stimulating the sympathetic supply, hyperpolarization with parasympathetic stimuli—are presumably associated with secretions of different salivary components and probably involve the flow of oppositely charged ions. Such secretory activity for Na^+ and for Cl^- are known in frog skin[91] and dog gastric mucosa,[92] respectively.[*]

This recently discovered system has not yet been studied pharmacologically. The existence of cells with dual innervation and oppositely directed electrogenesis, however, and of others that respond to both types of stimulation with the same electrogenesis of either sign probably accounts for the many varieties of results obtained in physiological and pharmacological studies of glands.

Cells That Respond with Dual PSP and a Spike

The heart. The electrophysiology of cardiac synapses has recently been considerably advanced.[93, 94] The pacemaker cells have a hyperpolarizing PSP developed by vagal excitation, and this electrogenesis slows or stops the rhythmic depolarizations that characterize the tissue but whose driving mechanism is unknown. Stimulation of the sympathetic, predominantly adrenergic and accelerator nerves increases the rate of this depolarization and thereby speeds the cardiac rate. The existence, to different degrees in different species, of cardiac acceleration action by the vagus nerve and of depressor action by the sympathetics[95, 96, 97] indicates a multiplicity of myocardial effector junctions with different types of electrogenesis such as are found in the salivary gland cells.[56] The electrophysiology of these aspects has not yet been studied in primary detail nor, indeed, is the innervation of the heart clearly known.[98]

A detailed analysis of cardiac pharmacology is beyond the scope of this paper. Several drug actions will be described, however, because of their more general bearing and because some of the experiments formulated in terms of the views in the present account fulfilled predictions.[99]

The vagolytic action of the classical cholinergic-blocking agents, atropine and curare, are well known. These inactivating drugs should produce no postsynaptic electrogenesis, and therefore they should not alter the cardiac rate. This is also the experimental finding. Hexamethonium blocks transmission at the intracardiac inhibitory vagal ganglion cells,[100, 101] but does not depress excitatory effects of vagal stimulation or of acetylcholine in the isolated heart of the rabbit.[101]

[*] Lundberg (personal communication) has found chloride transport in the salivary gland. Riker and Wescoe [92a] have presented interesting data on the pharmacology of the whole gland that accord well with the analysis of drug action outlined here. DFP increases the effectiveness of excitation through the chorda tympani, or by acetylcholine injections. Progressively larger injections of DFP, in spite of inactivating cholinesterase, lead to continuously increasing "spontaneous" secretion. These findings indicate that DFP is an activating drug, at least for the cholinoceptive membrane of the cells, unlike its initial role in the eel electroplax. The absence of an electrically excitable membrane component that is demonstrated in Lundberg's work leads to a relatively uncomplicated relation between the concentration of added drug and the secretory activity that it produces.

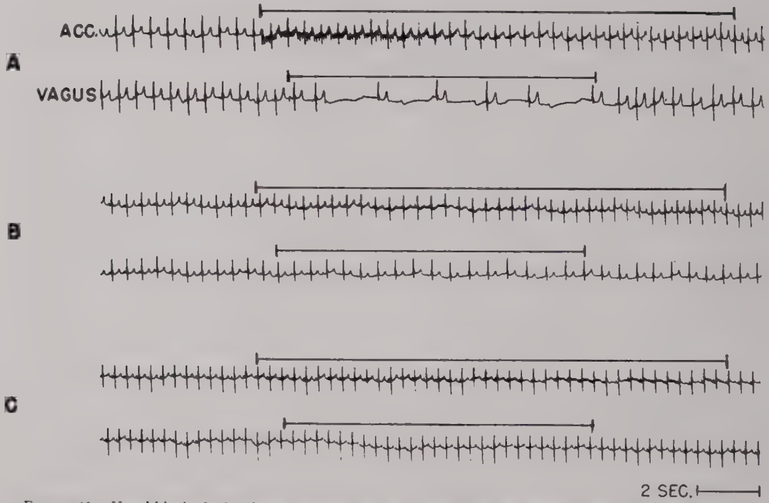

FIGURE 18. Vagal blockade developing earlier than the blockade to sympathetic stimulation in a spinal cat. The bars indicate the periods of stimulation. (*A*) Stimulating the cardiac nerve produced a relatively small effect, an increase of 20 per cent in rate. (*B*) Two minutes after injection of 3 mg./kg. succinylcholine. The basal rate increased about 10 per cent, but sympathetic stimulation again increased this by 20 per cent. Vagal stimulation became much less effective than before. (*C*) Two minutes after complete blockade was instituted by an additional injection (15 mg./kg.). The basal cardiac rate, now 40 per cent higher than originally, was not affected by stimulating either nerve.[99]

Acetylcholine slows and stops the heart by activating the intracardiac ganglia as well as the effector synapses.[100] Hyperpolarization of the pacemaker cells and cardiac inhibition by acetylcholine have been observed with intracellular recording.[93, 94] Recent experiments with succinylcholine[99] furnish some additional information on the pharmacology of cardiac synapses.

In unanesthetized cats with high spinal section, injection of 3 to 8 mg./kg. of succinylcholine leads to vagal blockade (FIGURE 18) and, somewhat later, to sympathetic blockade as well. As in clinical experience, the drug also produces tachycardia. If succinylcholine were to act on the cholinoceptive effector sites in the heart, the result would be a slowing of the heart. Succinylcholine therefore is probably much less effective than is acetylcholine on these hyperpolarizing synapses.* The site of the blockade is probably to be sought in the postsynaptic cardiac ganglion cells. These, normally excited by the vagal volleys, must have a depolarizing PSP that initiates postganglionic impulses to the effector junctions. Succinylcholine therefore probably depolarizes the synapses and inactivates their conductile electrically excitable membrane. This link being broken, transmission of the preganglionic vagal stimulation to the hyperpolarizing pacemaker membrane is prevented. Absence of an initial slowing of the heart is ascribable to the mode of application of the drug, systemic injection in the intact animal.†

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* This is also shown by the failure of very large quantities of succinylcholine to slow the heart.[102]

† Perfusion of the isolated heart of the cat with low concentrations of nicotine also causes vagal blockade without slowing the heart, whereas single injection of a large quantity produces marked inhibition.[100] Different results obtained with different types of application of a drug are also seen with the effects of nicotine on the ciliary ganglion as observed by reactions of the pupil. The explanation given by Perry and Talesnik[100] accords with the views developed in this paper.

Although adrenergic synapses are not generally acted on by cholinomimetic drugs, nevertheless the blockade of sympathetic cardiac actions indicates that succinylcholine affects these sites. Blockade probably comes about by the depolarization of these excitatory synapses and subsequent inactivation of local regions of the myocardial electrically excitable membrane. In the syncytial system of heart muscle, such local blocks could be instituted without interfering with conduction in most of the muscle. The depolarization, however, spreading, although attenuated, throughout the myocardium should heighten the excitability of the latter, and this should result in some quickening of conduction and also in the rate of the pacemakers. The tachycardia observed in the experiments can thus be accounted for.

In similar experiments, but on cats anesthetized with Nembutal (FIGURE 19), the total synaptic blockade is also produced by succinylcholine, but with a different overlay of detail. The order of blockading is reversed in the presence of Nembutal, that of the sympathetic innervation coming first. As may be expected, therefore, recovery from blockade occurs first at the vagal synapses. In addition, tachycardia does not develop. The nature of these effects of Nembutal is not known, but it may be connected with a depressant action of this drug upon cardiac muscle. The facts emphasize, however, the complex interrelations of drug actions and the need to evaluate these complexities in every given experimental situation.[*]

The concentration of succinylcholine required to block cardiac synapses of the cat is somewhat higher than that which causes paralysis.[103] Whereas 23 mg./kg. of the drug blocked vagal effects for about 1½ hours, however (FIGURE 17), about 50 mg./kg. are required to paralyze the rabbit for 1 hour.[104] Thus, the potency of the drug at the cardiac synapses of the cat appears to be intermediate between that at the neuromuscular junctions of the cat and the rabbit. Such differences, exhibited at a variety of synapses in the same animal or at homologous synapses of different animals, are common, and they indicate subtle variations in the molecular structure of the electrogenic membrane.[†]

Smooth muscle. The electrophysiology of the many varieties of smooth muscle is almost unknown, but it appears to differ considerably in different tissues. It is likely (Rosenblueth[106]) that some types of smooth muscle are not electrically excitable. Others generate spikes and therefore are conductile.[107, 108] Some uterine muscle is electrically excitable and conductile only when adequately supplied with estrone,[107, 109] and it becomes nonconductile in the anestrus phase, or when treated with progesterone. Under these circumstances the muscle responds either only with graded electrically excitable responses (Grundfest[5]) or is perhaps excited to local contractions only by PSP.

It is not known whether all smooth muscle fibers are innervated or whether a large portion of these in any given tissue are excited only by circulating chemical agents. Likewise, it is unknown whether those muscle fibers that are innervated are each supplied by nerves of opposite actions (Furchgott[61]), or whether the antagonistic effects such as relaxing or contracting blood vessels

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[*] Preliminary experiments indicate that *d*-tubocurarine lifts sympathetic blockade, but not the vagal, suggesting that this drug competes with succinylcholine at the adrenergic cardiac synapses.

[†] Vagal cardiac synapses in the rabbit are almost totally insensitive to succinylcholine (Grundfest, unpublished). An interesting mechanism, apparently genetically determined, renders some rabbits insensitive to atropine (Ambache[105]). This is caused by the presence of an enzyme that destroys the drug.

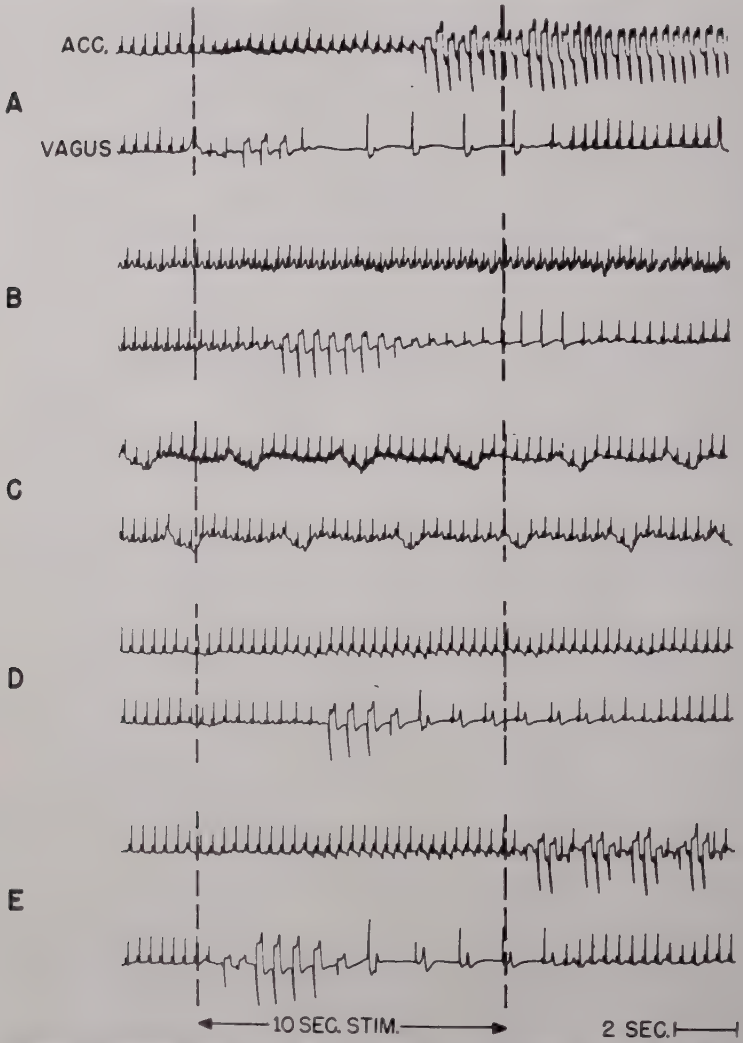

FIGURE 19. Reversible cardiac synaptic blockade induced by succinylcholine in the cat anesthetized with Nembutal. Upper trace of each set shows the effects of stimulating the inferior cardiac nerve; the lower shows the results of stimulating the vagus nerve. (*A*) Prior to intravenous injection of succinylcholine. (*B*) Five minutes after 8 mg./kg. succinylcholine, sympathetic blockade was established, but not vagal. (*C*) Five minutes after an additional injection of 15 mg./kg., vagal blockade was almost complete. (*D*) Thirty minutes later, stimulation of the vagus nerve again produced marked effects, but sympathetic blockade was still in force. (*E*) One hour later, blockade almost completely lifted.[99]

or other structures are caused by singly innervated muscles of opposite structure and function, as found in circular and longitudinal layers (*cf.* Goodman and Gilman[110]).

A few relatively simple assumptions, however, derived from electrophysiological knowledge of other structures appear adequate to account for much of the data on the pharmacology of smooth muscle.

Contraction is assumed always to be associated with depolarizing electrogenesis, produced either at innervated synaptic sites or at noninnervated "receptor" regions sensitive to specific activating agents. This electrogenesis may occur without the presence of spikes, as in the "slow" muscle fibers of the frog, or with spikes. On the other hand, relaxation, at least in some muscle fibers, is assumed to be associated with hyperpolarization, again either at synapses or at receptors. This does not rule out the presence in certain structures of differently oriented muscle fibers whose contractions yield vectorially different responses. The assumption of both "inhibitory" and "excitatory" electrogenesis in the same fibers appears to be called for by the common observation that relaxation of many smooth muscles is best evidenced when the muscle is first placed in a state of "tonic" contraction.[61] A given excitant is capable of activating either depolarizing or hyperpolarizing electrogenic sites in different types of muscle. Thus, one relaxed cholinergically may be contracted adrenergically. It may also be possible, however, that the same muscle fiber has oppositely directed, but similarly excited electrogenesis, the net mechanical effect being determined by the algebraic summation of the resultant potentials.

Hyperpolarization of itself probably does not affect the contractile mechanism. It is likely, however, that stretch of smooth muscle causes some depolarization and a contractile response. This assumption implies that mechanotransducer membrane[4] also exists at least in some muscle fibers. Muscle fibers under certain conditions become exceedingly sensitive to mechanical stimuli[111] and can discharge in proportion to the degree of stretch.[112] Whether the postulated mechanotransducer membrane is separate from the synaptic or receptor structure or is part of one or both of these[4] is immaterial for the present.*

The occurrence of receptors in greater variety than merely the adrenoceptive or the cholinoceptive is well documented. Indeed, smooth muscle indicates rather clearly, although this is also to be seen in other systems, that the single categories probably encompass a number of variants. Thus, for example, epinephrine, norepinephrine, and isopropylarterenol have relative effects for contraction of some vascular muscle in the ratio 1:1:0.01. For relaxation of the same muscles the ratio is entirely different, 1:0.02:4.[61] Similar differences are found in other synaptic systems. The relative effectiveness of gallamine (Flaxedil) and *d*-tubocurarine varies greatly for different muscle synapses.[114] The different action of decamethonium on the muscle and ganglionic synapses[78] has already been noted. Likewise, the relative effects of epinephrine and norepinephrine on cerebral blood flow and on oxygen consumption of the brain differ markedly from their vasopressor action.[115]

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* Of interest in this connection, however, is the sensitivity of sensory receptors to synaptic drugs. The mechanotransducer membrane of the stretch receptors of crayfish is also stimulated by acetylcholine.[88] Cutaneous sensory discharges are elicited in the cat by acetylcholine, nicotine, and α-lobeline. This excitation, furthermore, is blocked by hexamethonium or *d*-tubocurarine.[113]

The effects of blocking agents acting on the specific varieties of the chemosensitive electrogenic regions can be easily accounted for on the basis of the foregoing. The inactivation of depolarizing, contraction-producing receptors would enhance or unmask relaxant responses. The contrary would be the case with agents that block hyperpolarizing relaxant electrogenesis. Competitive actions will be discussed below.

Agents activating specific sites could develop complex effects, depending upon the variety of electrogenesis caused (1) by the drug; (2) by the presence or absence of electrically excitable membrane; and, if the latter is present, (3) by its specific properties. An agent activating only hyperpolarizing electrogenesis might lead to relaxation and inhibition of contraction developed by other means, or to a decrease of excitability to various degrees depending upon the relative magnitudes of excitatory and inhibitory electrogenesis. In fibers devoid of electrically excitable membrane, activation of depolarizing electrogenesis would yield "tonic" contractions and resistance to inhibitory stimuli. Excitation of depolarizing responses in fibers with spike-producing membrane might lead to vigorous contractions of the muscle followed by relaxation and block to further excitation as inactivation of the electrically excitable membrane developed. Competitive action by blocking, inactivating drugs might restore excitability.

The pharmacological literature is replete with examples of these various effects. Despite the lack of much relevant and necessary data, the foregoing speculative discussion has been included to serve a twofold purpose: to stimulate detailed electrophysiological research on these interesting and important systems, and to suggest the formulation of pharmacological experiments that might serve as critical tests. The mode of excitation of smooth muscle by its nerves or by drugs and the modes of interaction of drug effects suggested in this section has a basically different orientation from Rosenblueth's[106] formulation of the same problem. It is postulated here that transfer of action across the cell membrane to the contractile effector mechanisms is mediated by postsynaptic (or receptor) electrogenesis, depolarizing and hyperpolarizing. This view encompasses cases in which electrically excitable, conductile membrane may also participate in the transfer. The principles employed in this formulation also avoid a dichotomy between transmission at "neuroeffector" junctions and at peripheral synapses. The formulation, indeed, stresses their fundamental similarity in the face of diversities in pharmacological properties.

Classification of the Action of Drugs on Synapses

Before proceeding to an analysis of the central actions of drugs it is useful to recapitulate the conclusions of the foregoing sections and to deal with some of the secondary complications produced by drugs.

NOMENCLATURE OF SYNAPTIC DRUGS

Acetylcholine, which depolarizes those synapses endowed with depolarizing PSP (electroplax, muscle fibers, and autonomic ganglion cells), hyperpolarizes cardiac muscle as do the cholinergic nerve impulses (FIGURE 13). Therefore acetylcholine, a "depolarizing" drug for some synapses, is a "hyperpolarizing" and hence "inhibitory" drug for others. On the other hand, succinylcholine apparently acts as a "depolarizing" drug on the adrenergic cardiac effector synapses and, similarly, on the presumably cholinergic synapses of the intracardiac ganglion cells of the vagal system. Likewise, in salivary glands, acetylcholine may produce depolarization or hyperpolarization, depending entirely on the type of cell involved. Adrenalin can produce the same effect.[56]

For these and other reasons it has been suggested[5] that certain drugs should be termed "synapse activators," as they have the property of exciting postsynaptic membrane to generate whatever electrical response that structure is capable of developing. The electrogenesis so evoked then acts upon electrically excitable membrane if this is present. Paralleling this term, the "nondepolarizing, competitive, antagonistic" agents are designated as "synapse inactivators." Data have been presented above, specifically for the synapses of electroplax in the eel, and in the muscle, to show that reversible or irreversible block of postsynaptic electrogenesis is the primary effect of these drugs. The nomenclature suggested has not only the purpose of describing more precisely the nature of the observed actions of drugs on postsynaptic membrane.* It also helps to clarify the effects exerted by the drugs in systems that are complicated by the presence of both depolarizing and hyperpolarizing synaptic electrogenesis and in the interplay of these potentials on the spike-generating, electrically excitable membrane.

TRANSMITTERS AND THE POTENCIES OF SYNAPTIC DRUGS

The theory of synaptic actions of drugs developed in this paper bases itself on the concept that synaptic membrane is only chemically excitable. Therefore, it can add nothing to the fact that certain naturally occurring substances can excite certain types of synapses or produce certain types of overt effects, since this information is a starting point. In so far as the theory attempts to draw a general picture of mechanisms, however, several conclusions of a general nature can be voiced.

With the data of the previous sections in mind, it seems safe to suggest that specific activating or inactivating performance by various drugs at various types of synapses is only relative. In large measure, attention is usually paid to drugs that are highly effective with one or another type of junction, the less dramatic products being discarded. The search for such "specific," that is, high-potency compounds has been nowhere better illustrated than in the attempt to devise or discover more effective or more specific paralyzants or central nervous agents. While such compounds are very useful, the concentration of attention on one or another group of synapses abets a tendency to consider each minor variety of synaptic complexity as *sui generis*, not susceptible to generalized analysis. This fragmentation may hinder the development of more adequate theory in synaptic pharmacology which, in turn, may slow the successful development of drugs on the basis of rational approaches. The same considerations suggest that inflexibility in viewing as synaptic drugs only those compounds that have extraordinarily high potency tends to hamper pharmacologic theory. As TABLE 1 shows, compounds differ many-fold in potency, but their action, when exerted, is the same. Other examples have also been cited.

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* An important and very useful textbook of pharmacology[110] classifies succinylcholine with "other curariform drugs," which include the truly curariform agent gallamine (Flaxedil). Decamethonium is grouped with tetraethylammonium and hexamethonium. Ample data already cited indicate that both succinylcholine and decamethonium are in general "depolarizing," synapse-activating drugs, and that these drugs should have been listed, in the authors' grouping, with the choline compounds. These remarks are not directed to point out a flaw, flaws being inevitable in a comprehensive work. They are intended to emphasize the absence of general agreement on the nomenclature and classification of drug actions that the present suggestion seeks to remedy.

A somewhat similar conclusion evolves with respect to the transmitters. These substances, not all of which are yet known, have high potency of action. Their action, however, involves also the interaction of the transmitter with a labile structure of the postsynaptic membrane. The extraordinary fact of specific electrogenesis and the reproducibility of it during the lifetime of the system bespeak highly organized structure. However, the fact that this electrogenesis can be affected by a great variety of drugs, that some of these act more on one synaptic membrane than on another, that these actions can be of one type or of another, and that various kinds of interaction take place indicate that this organized structure is complex. To use a chemical analogy, even the relatively simple steroids and hormones are profoundly modified by small structural changes.

If the view proposed here is correct, the "muscarinic" and "nicotinic" subgroups of the cholinergic system can be considered as operating through a fundamentally similar mode of synaptic transfer and as subjected to basically similar types of drug action, although certain anatomical, physiological, and pharmacological relations distinguish one class from the other. It is furthermore unlikely that all synaptic membranes of either subgroup have the same molecular structures in a given species. Moreover, homologous synapses of different species do not necessarily have identical properties.

Competitive Antagonistic Actions

The kinetic theory. The concepts developed in this sphere about 25 years ago by A. J. Clark[116, 117a] from analogy with mass action principles in enzyme chemistry still appear to be valid today.[61, 117b] The competitive, antagonistic effects of synapse-activating and -inactivating drugs are well illustrated[118] in FIGURE 20. A quantitative description of a given action, however, would involve, if the view advocated here is correct, the factors of synaptic drive, the relative combining power of the synaptic membrane with one drug and the other, the amount of synaptically excitable membrane, and secondary effects of each drug. The simple synaptic system of the eel electroplax exhibits competitive antagonism,[12, 84] and this preparation could be a useful tool for quantitative analysis of various factors. Translating this information to other synaptic systems, however, would require taking account of the specific properties of their various electrogenic components.

Underlying mechanisms. It may be assumed that the molecular electrogenic components of electrically and synaptically excitable membrane are essentially all variants of a common structure type. Some insight into the processes underlying synaptic inactivation can then be gained from the similar effects

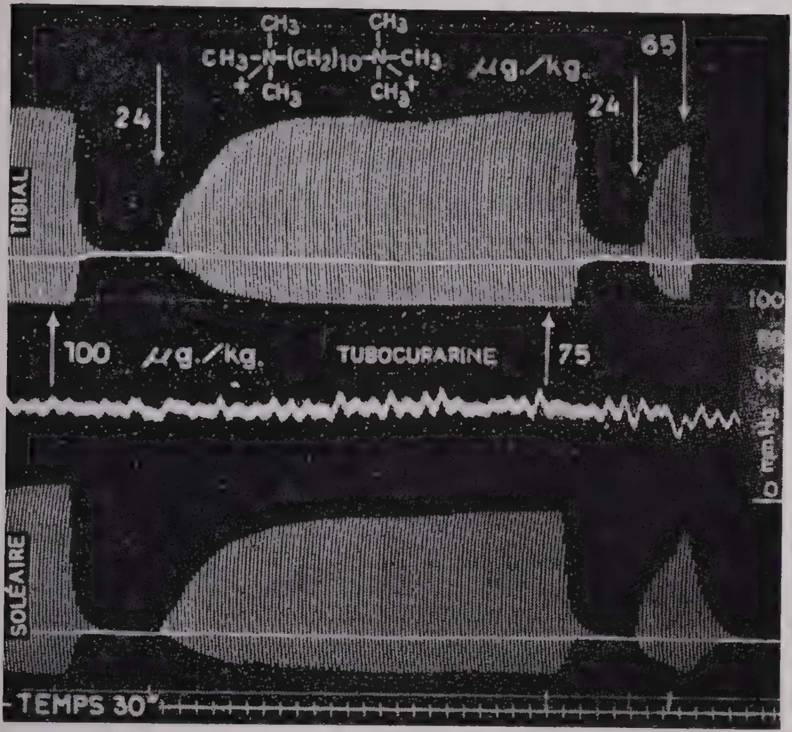

FIGURE 20. Interactions of *d*-tubocurarine and decamethonium in neuromuscular transmission to the tibialis and soleus muscles of cat.[118] The injection of *d*-tubocurarine (100 μg./kg.) blocked transmission to both muscles equally. "Decurarization" by C_{10} (24 μg./kg.) was more rapid in the tibialis, and a subsequent injection of *d*-tubocurarine (75 μg./kg.) was less effective in this muscle. A large injection of C_{10} (65 μg./kg.) caused more rapid "decurarization" in the tibialis, but also a more rapid inactivation. Middle tracing is of blood pressure.

which the inactivating drugs produce on the electrically excitable membrane. These drugs convert the explosively responsive membrane of the eel electroplax into one that is gradedly responsive (FIGURE 21), although more slowly than they block synaptic electrogenesis (FIGURE 5).[12] At maximum, the graded response is at first as large as the spike, but it subsequently decreases and the membrane eventually becomes unresponsive. Similar, but reversible, effects develop during refractoriness or depolarization (FIGURES 12 and 14) in all electrically excitable membranes thus far studied for this phenomenon,[2, 14, 119] and were obtained, but not recognized, by earlier observers.[5]

The probable cause of graded responsiveness is a change in the thresholds of the electrically excitable electrogenic units,[5] and a subsequent decrease in their number. All-or-nothing responsiveness probably denotes that the reactive units ("valves," "carriers," or whatever they may be) all have rather similar thresholds. Increased spread in the distribution of these thresholds would permit only a portion of the units to become excited by the local circuit

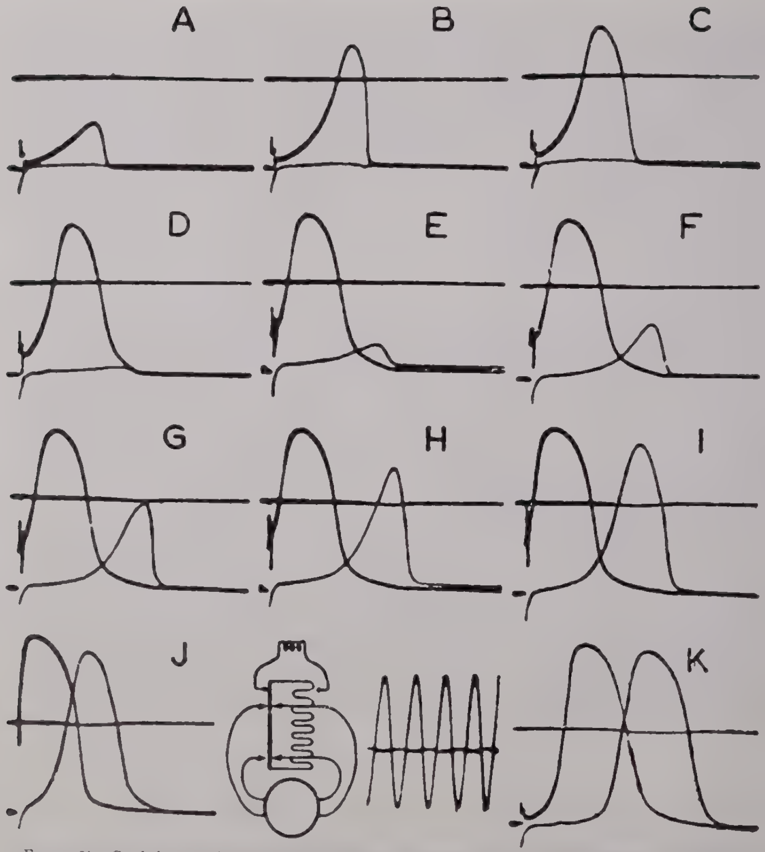

FIGURE 21. Graded responsiveness following chemical treatment of the eel electroplaque.[12] The initial responses of the cell to a direct stimulus are seen in *K*. After poisoning the cell with 1 mg./ml. eserine, the stimulus evoked only a small response at the proximal site, nothing at the distal site. Increasing strength of stimulation increased this response (*B* to *D*), still with no distal activity. The latter began to develop with stronger stimuli, showing the full range of gradation (*E* to *J*), but the response at the proximal site remained essentially unchanged, although arising with briefer latencies. The responses in *J* are almost of the same amplitude as are the spikes of the unpoisoned cell *K*. The resting potential remained unchanged throughout. Calibrations 100 mv. and msec.

of activity. In this condition, decrementless conduction, associated with all-or-nothing responsiveness, becomes impossible, although a strong external stimulus can evoke a maximal response as long as the number of electrogenic units remains unchanged.*

Transposing this analysis to the case of synaptic membrane treated with an inactivating drug, a fixed quantity of transmitter such as that probably released with a single presynaptic volley would find some of the electrogenic postsynaptic units unresponsive. Thus, the PSP should and does initially become smaller[72] and transmission is slowed and then blocked. Repetitive neural stimuli, however, or the combined actions of a neural volley and of synapse-activating drugs, should and do raise the amount of postsynaptic electrogenesis to the point of lifting the blockade (FIGURE 20). Prolonged action of the inactivator drug should and does depress the maximal amplitude of the PSP and eventually causes a blockade that cannot be overcome by synapse-activating compounds. This interpretation can account also for the progressive shifts in the concentration-action curves for smooth muscle exposed for different times and to different concentrations of various inactivating drugs (see FIGURES 1 and 2 in Furchgott[61]).

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* Poisoned eel electroplax that respond gradedly to a locally applied stimulus develop a spikelike, apparently all or nothing response to a threshold stimulus delivered simultaneously to the entire excitable surface.[120] The stimulus becomes effectively regenerative as the electrogenesis of units activated at "threshold" simultaneously in different "unit areas" of the cell-membrane sum with each other.

Secondary Effects of Synapse-Activating Drugs

Like the inactivating drugs, the synapse-activating drugs also exert secondary effects, sometimes on the electrically excitable membrane, but more commonly on the chemically excitable components. The writer's grasp of neuropharmacology cannot encompass all the perturbing effects that becloud studies in this field. Nor does he feel that in the present state of pharmacological knowledge and of its correlations with electrophysiology it is useful to touch on more than the prominent types of secondary actions.

Electrically excitable membrane. The eel electroplax which are first appropriately treated with an inactivating drug are not depolarized by the synapse activators. The electrically excitable membrane is therefore not subjected to the secondary actions of depolarization. The combination of the drugs, however, makes this membrane absolutely unresponsive much more rapidly than does the inactivating drug in isolation (Altamirano *et al.*[12] and unpublished experiments of that series).

Tachyphylaxis. The decrease or disappearance of the action of a given drug may be ascribed in part to changes in the sensitivity of the postsynaptic membrane. Thus (FIGURE 12), the PSP developed in the eel electroplaque by a weak neural stimulus becomes smaller under prolonged exposure of the cell to acetylcholine, although a strong neural volley elicits a maximal and full-sized PSP. Decreased sensitivity to acetylcholine has been studied in autonomic ganglia.[81] The ganglion subjected to a constant amount of the drug gradually repolarizes and transmission also returns. It is depolarized and blocked again by an increased quantity of acetylcholine. Prolonged interaction between the synaptic membrane and the drug, however, results in irreversible loss of synaptic electrogenesis. This sequence may account for somewhat similar effects observed in frog muscle.[80]

Dual actions. Other and probably related secondary effects involving changes in sensitivity are seen in the eel electroplax. DFP, which at first behaves as a synapse inactivator (TABLE 1), subsequently depolarizes the cell. Whether this is due to an action on altered postsynaptic membrane or represents permeability changes in the electrically excitable membrane is not known.

Some diphasic actions of drugs, excitatory at first and then depressant, may also be analyzed in simple synaptic organizations. One type of these is seen particularly in cholinergic transmission. Synapse inactivation by eserine or DFP is at first masked by the protection that the drugs offer to the transmitter. The initially increased excitability to neural volleys produced in the eel electroplax by eserine (FIGURE 4) is, indeed, perhaps the best evidence at present that this electric organ has cholinergic transmission. As inactivation of synaptic electrogenesis progresses, depression and blockade of transmission ensue.

The activating drugs, summing their electrogenesis with that of the transmitter, also increase excitability of the eel electroplax to their neural stimuli.[12] The subsequent depression as depolarization affects the spike-generating membrane might be lacking in cells that have no electrically excitable component. In more elaborate synaptic complexes the types of diphasic actions may be expected to exhibit still more marked effects exerted through the variety of electrogenic mechanisms and synaptic pathways. The complexity of synaptic organization of the central nervous system offers many opportunities for diphasic actions and these prominent features of central activity are illustrated later (see also Purpura[7]).

Alteration of the response with the frequency of presynaptic excitation may also be considered as a secondary effect of the interplay of excitatory and inhibitory actions in the central nervous system. In simple systems this effect also occurs (FIGURE 6). The depression of postsynaptically effectuated electrogenesis of the spike at high frequencies of stimulation develops in the face of sustained capacity for transmission from the presynaptic nerve to the postsynaptic membrane, but in its over-all manifestations transmission is blocked. This type of effect has been long known as the classical Wedensky phenomenon. The synapse-activating drugs, by adding to the synaptic depolarization caused by the nerve impulses, should and do increase Wedensky inhibition.[106] Other types of action, such as the exhaustion of transmitter, or its tachyphylactic effects on the postsynaptic membrane, may also participate, all producing "fatigue" of synaptic transmission. Rosenblueth[106] has described many details of what appear to be effects of such a type in neuromuscular and ganglionic transmissions and the interactions of these with drugs, but space precludes their analysis in terms of the present concepts.

Effects produced by denervation. The wide variety of phenomena associated with denervation[121] will be dealt with only briefly and, primarily, to suggest several areas of research. The atrophic changes of denervated postjunctional cells are particularly clear in muscle, but also occur elsewhere and, as transneuronal degeneration, in the central nervous system. The rarity of the latter may be accounted for[122] by the difficulty of achieving complete denervation in the central nervous system. The nature of these profound effects is unknown. Apparently unexplored is the possibility that the absence of synaptic excitation, eliminating or diminishing the frequent changes in permeability of the membrane such as occur during responses, also removes a portal for the entry of material from the exterior into the cell. This deficiency, if it is a factor, may be expected to be more pronounced for those cell types that are normally activated by an evanescent transmitter.

Altered excitability of the postsynaptic membrane to drugs is exemplified by the reaction of the denervated end plate to *d*-tubocurarine (Jarcho *et al.*[123]). The drug, although in rather high concentration, activates the end plate electrogenesis and evokes spikes and contractile responses. Heightened excitability to the normal transmitter or its analogs occurs in many systems. Denervated muscle end plates become about a thousandfold more sensitive to acetylcholine.[75]

In man, peripheral denervation is accompanied by a phenomenon that becomes distressing and usually persistent after re-innervation. This resembles the complex of Raynaud's disease, and denervation may, indeed, be one etiological factor in that disease. Changes in sensitivity of the synapses or receptor sites of vascular smooth muscle are probably involved. Autonomic denervation alone, however, does not lead to the symptoms. The possibility that the sensitivity of the vascular smooth muscle is affected by the loss of dorsal-root vasodilator fibers and thereby becomes abnormally responsive to circulatory agents has not yet been explored.*

Reversal of responses. During pregnancy the uterus of the cat is contracted by epinephrine, but in the nonpregnant animal the response is a relaxation. Corpus luteum[123a] and progestin[123b] also cause the reversal that obtains in pregnancy. The reversal of response bears a striking similarity to the effects of hormones on electrical excitability mentioned earlier. Dale[71] suggested that the uterus had 2 oppositely directed muscles, one or the other being more sensitive to epinephrine in the 2 conditions. It appears to be more likely, however, that a single set of muscles develops these opposite types of response.[106] If the first view is correct, the hormones increase the sensitivity of the electrogenic chemoreceptors to epinephrine in one type of muscle and probably depress that of the second. The same action, but exerted on oppositely electrogenic membrane of the same fiber, could account for the effect if it operates through a single type of muscle. Another possibility is that hyperpolarizing electrogenesis is converted to depolarizing electrogenesis by the action of the hormones. Whichever mechanism may be involved, change in excitability or in the type of electrogenesis, the fact that hormones can affect the response may be an important determinant in other synaptic systems as well.

INTERACTIONS OF SYNAPTIC DRUGS

Our ignorance of the molecular structure of excitable membrane, of its mode of electrogenesis, and of the structural reactions of drugs with the membrane is often illustrated experimentally by unforeseen interactions of drugs. It was shown earlier (FIGURES 18 and 19) that the effects of succinylcholine upon cardiac synapses are different in the presence or absence of Nembutal, and that succinylcholine appears to activate adrenergic cardiac synapses. Epinephrine also has adjuvant, perhaps weak excitatory actions on the cholinoceptive membrane of muscle end plates (the Orbeli effect[124]). Epinephrine may also behave as an inactivator.[125] At muscle synapses it blocks the depolarizing effects of decamethonium (FIGURE 22), but only when applied together with or

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* Experimental embryology and morphology show, with many examples, the importance of neural elements in the differentiation or regeneration of organs.

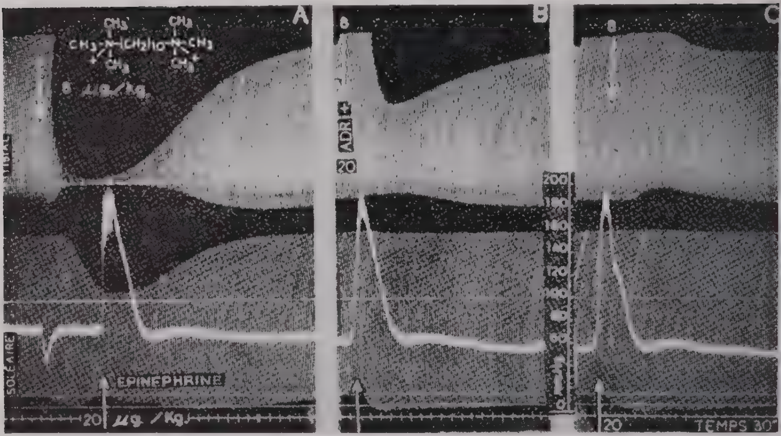

FIGURE 22. Antagonism between epinephrine and decamethonium.[118] (*A*) Epinephrine (20 μg./kg.), given about 7 minutes after C_{10} (8 μg./kg.), did not lift neuromuscular blockade. Note that the soleus responds much more slowly and minimally to the decamethonium than does the tibialis (as also seen in FIGURE 20). (*B*) When the two drugs are injected together the neuromuscular blockade is diminished in the tibialis and abolished in the soleus. (*C*) Epinephrine injection precedes that of C_{10}. The response of the tibialis to the latter drug is diminished still more and appears very late. Superposed blood pressure tracings show the transient fall on the first injection of C_{10}, and high rises with each injection of epinephrine.

prior to the administration of that drug.[118] Heparin and synthetic heparinoids act as anticurare agents[126] and, as will be shown below, this effect can also be demonstrated in the central nervous system.

Effects of Drugs on Central Synapses

GENERAL CONSIDERATIONS

Under the conditions of enormous structural and functional complexity that prevail in the CNS it may be expected that the actions of drugs will be complicated, even if their general behavior with respect to synapses also applies to those of the CNS. The probability that a number of synaptic transmitters operate in the CNS[127] would further suggest that the varieties of synaptic drugs and of the modes of their interactions might be rather large. Factors already noted in peripheral synapses, such as differences in relative potency of synaptic drives, might play an even more important role in the CNS with its many and complex interconnections (Purpura[7]). Nevertheless the central synaptic actions of drugs can be analyzed with considerable success, although the over-all reactions of the CNS or of the whole organism are probably too complex for a complete analysis of their mechanisms with present-day knowledge.

SYNAPSES OF THE SPINAL CORD

Electrophysiological data. Microelectrode studies[23, 45, 53, 64] have demonstrated the mechanisms of several properties that are prominently displayed in the spinal cord. The final common path of the motoneurons is subjected to many excitatory and inhibitory drives[66] that are manifested by excitatory and inhibitory synaptic electrogenesis. An important factor is the existence of specific paths for inhibition.[128, 129] This function is exercised by "Renshaw cells," interneurons whose existence in the CNS was postulated by Hunt in 1921.[130] By virtue of their capacity to fire repetitively at high frequency, Renshaw cells can develop long sustained hyperpolarization at the motor neurons.[53] Other interneurons, of as yet unknown function, can also maintain rapidly repetitive discharges.[64]

Action of drugs. The motoneurons of the cat appear to be relatively resistant to drugs.[53, 63] Eccles and his colleagues have concluded that these synapses do not belong to either the cholinergic or adrenergic groups. These synaptic systems, however, need to be examined in greater detail before this conclusion is accepted:

(1) For instance, the postsynaptic membrane of motoneurons may have only a relatively higher threshold to the synaptic drugs used thus far.

(2) Also, the probable presence of glial sheaths around the motoneurons[131] may contribute to make their synapses rather inaccessible to drugs.* A somewhat analogous effect has been observed in the case of the inhibitory interneuronal Renshaw cells.[63] Prostigmine does not affect these when the drug is injected into the spinal blood supply, but it does exert an action when injected directly into the substance of the cord.

(3) A further complication may arise from the presence of both excitatory and inhibitory synapses. If these are activated to approximately equal degrees by a drug, the net electrogenic action may be negligible and overt effects absent. Eccles and his colleagues assume, probably with good reason, that opposite types of electrogenesis are evoked by different transmitters. Such types might, at least at some sites, be generated by the same transmitter.[3] Interaction in normal transmission might be minimized by the restriction of the transmitter to the local sites of their release,[2, 5] while the application of drugs would have widespread action.

The activation of cholinergic synapses of the Renshaw cells by various cholinomimetic agents and their inactivation by blockers of cholinergic synapses have been demonstrated by Eccles and his colleagues[63] (see also Fatt[133]). Close arterial injection of acetylcholine into the spinal cord of the cat evokes an outburst of impulses. These are superimposed on a fluctuating potential that may be the reflection of synaptic electrogenesis produced by the drug. The effects are considered to arise from excitation of interneurons.[134] Epinephrine, like acetylcholine, augments excitability in the spinal cord, probably also through an action on the interneurons. A selective effect on extensor reflexes[135] has been questioned.[136] The spinal action of *d*-tubocurarine, augmenting excitability, is also considered to be effectuated through the motoneurons.[135] As shown below, this probably involves preferential blockade of spinal inhibitory synapses.† Strychnine also blocks inhibitory synapses.[53] Many of the electrogenic effects of synaptic drugs have not as yet been directly demonstrated, but the resultant overt actions are in accord with the principles outlined in the foregoing sections.

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* This may be analogized to the explanation by Dale and Gaddum[132] of the failure of atropine to block the effects of nerve stimulation, when vasodilation by acetylcholine is blocked.

† When injected into a preparation containing a subconvulsive dose of strychnine, *d*-tubocurarine potentiates the strychnine, convulsive electrical activity then developing in the spinal cord.[146a] Thus the action of *d*-tubocurarine in the spinal cord of the cat appears to be similar to that of strychnine; that is, blockade of inhibitory electrogenesis. Little or no inhibitory PSP may therefore be expected in the intracellular studies of curarized spinal cord preparations.

SYNAPSES OF THE CEREBRAL CORTEX

Electrophysiological Data

Experimental difficulties for intracellular recording from cortical neurons have been overcome by Phillips.[137, 138] Cells, identified as pyramidal by their activity on antidromic stimulation of the tracts in the medulla, undergo rhythmic depolarization which discharges spikes at a critical firing level of about −50 mv. Bursts of activity may be associated with subsequent prolonged membrane polarization at levels of −60 mv. or more. Similar although less clear-cut correlations appear in the work of Li and his colleagues.[139-142a] The spikes that arise by antidromic invasions rise abruptly without the slower prefatory depolarization of "natural" activity. At other times stimulation of the pyramidal tract evokes late responses in some cells, and these are associated with the initial depolarization. These patterns suggest that the latter type of response, as well as the "natural," occur through synaptic excitations, prefaced by a depolarizing PSP. In the case of antidromic stimulation the synaptic activity probably develops through interneurons discharged by recurrent collaterals of active Betz cells. Even after an antidromic direct response there is frequently a long phase of hyperpolarization, indicating that inhibitory PSP also are initiated by the interaction of recurrent collaterals and Renshaw cell analogs in the cortex.* It is possible, however, that spinocortical afferents of the pyramidal tracts are also involved.[143] Stimulation of the cortical surface also evokes responses usually preceded by depolarizing potentials and followed by hyperpolarization. This sequence is especially evident when the stimulus is applied to a region of cortex some distance from the site of the recording electrode. The strength of the threshold stimulus is rather independent of the distance or of the sign of the stimulus (see FIGURE 4 in Phillips[188]).

The data therefore indicate strongly that the pyramidal cells of the cortex of the cat develop both excitatory and inhibitory PSP; that antidromic stimulation also excites interneurons capable of exerting both electrogenic actions on the Betz cells; that these interneurons are probably stimulated through the recurrent collaterals of the pyramidal neurons; and that surface excitation of the latter can develop by electrical stimulation of presynaptic fibers at the cortical surface. The same conclusions were reached independently by Purpura and myself[48, 144, 145a,b] from studies of the surface negative potential recorded from the cortex as evoked by a variety of stimuli (FIGURE 7). This potential[146] has been identified as the response of apical dendrites of the pyramidal cells.[147, 148] It is eliminated by *d*-tubocurarine when the synaptically evoked activity in the pyramidal tracts[149] is similarly eliminated (FIGURE 7, C4-6). Stimulation of the tract appears to activate the dendrites through the intervention of interneurons, excited by recurrent collaterals (FIGURE 7, C1-3), since the surface positive component of this response is dual, the second portion disappearing after curarization. When *d*-tubocurarine blocks dendritic responsiveness, the dendrites are not electrically excitable[49] either to direct cortical shocks (FIGURE 7A), to stimuli under the cortical surface (FIGURE 7B), or through the local circuit of activity (FIGURE 7, C1-3). Thus, as mentioned earlier, the dendrites have the properties of an electrically inexcitable postsynaptic membrane. Our findings confirm the conjecture of Eccles[150] that the surface negative potential is a PSP of dendrites. Data, including the production of electrical silence of the cortex after curarization and other findings, indicate that the EEG is primarily a manifestation of dendritic potentials.

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* Albe-Fessard and Buser[142b, c] also recorded excitatory and inhibitory PSP in cortical cells. The hyperpolarizing PSP may be of long duration, of relatively large magnitude, and capable of inhibiting a train of "spontaneous" cell discharges.

Action of Drugs

Action of d-*tubocurarine.* The synaptic blockade of the cortical dendrites described in the foregoing indicates that their synapses behave in the same way as do peripheral synapses of the cholinergic system. Abolition of electrocortical activity was first demonstrated in this laboratory by Ostow and Garcia,[151] and was confirmed on the perfused cat brain preparation[152] and in the present work (FIGURE 7). Only a few drugs have as yet been used in the current study, and that has extended only to a few types of synaptic systems. Nevertheless, the data already available permit a number of analyses of cortical organization and drug action.

The concentration of *d*-tubocurarine required for cortical synaptic blockage is higher than that needed at neuromuscular junctions, but it has been shown that the effect does not depend on secondary changes in blood pressure, nor on direct actions of the drug on the dendritic excitability. Intracarotid injection of about 0.5 mg./kg. (or about twice the dose used in paralysis preparatory to electroshock therapy) is adequate to block the dendritic synapses.

Interactions of d-*tubocurarine and heparin: enhancement of dendritic potential by* d-*tubocurarine.* An instructive interaction of drugs was observed by chance during the simultaneous study of the effects of *d*-tubocurarine on blood pressure and on dendritic potentials (FIGURE 23). In the presence of heparin, *d*-tubocurarine does not block dendritic responses, but enhances them enor-

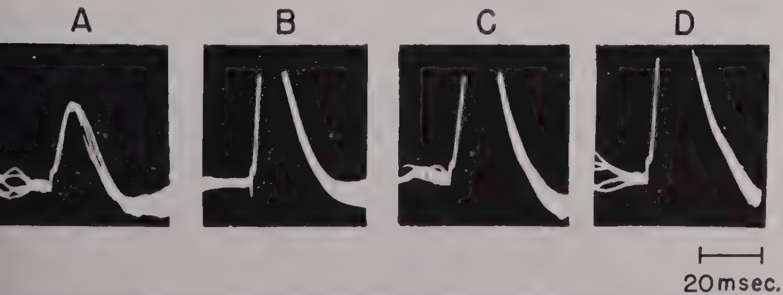

FIGURE 23. Interaction of heparin and *d*-tubocurarine at dendritic synapses of the cat cerebral cortex.[146a] The surface negative response was recorded from the suprasylvian gyrus about 1 mm. from the site of a surface stimulus. (*A*) Two and one-half hours after 50 mg./kg. heparin i.v. (*B*) Five minutes after 3 mg./kg. *d*-tubocurarine i.v. (*C*) Five minutes later, after additional 5 mg./kg. *d*-tubocurarine. (*D*) Ten minutes after *C*, and 5 minutes after another injection 10 mg./kg. Five consecutive traces are superposed in each set

mously. In part, this surprising effect is ascribable to the anticurare action of heparin and of synthetic heparinoids,[126] which was not known to us at the time.

Selective actions on different synapses. While this competitive action can account for the failure of *d*-tubocurarine to block the heparinized synapses, the enhancement of the responses seen in FIGURE 23 indicates an additional mechanism. The finding suggests that heparin protects the excitatory synapses from blockade by *d*-tubocurarine, but does not so protect the inhibitory synapses. Successive injections of *d*-tubocurarine, blocking increasingly larger numbers of the latter, can account for the progressive increase in the effectiveness of excitatory synapses as more drug is injected.*

The differential and greater affinity of *d*-tubocurarine for inhibitory synapses has also been demonstrated in other ways, as described below. It accounts also for the strychninelike action of topical application of *d*-tubocurarine to the cerebral cortex[153, 154] (confirmed by Purpura and Grundfest, unpublished experiments). Strychnine, which depresses synaptic excitation, likewise appears to act more strongly in blockading inhibitory synapses of the cortex, as it also does in the spinal cord. The early enhancement of dendritic responses by topical or systemic application of low concentrations of strychnine[155] appears to be due to this selective action. The convulsant electrocortical activity ensues when the excitability of dendrites is actually depressed by the drug,[7] and the form of the responses, usually diphasic and in either sign, indicates that these are produced by an overbalancing release of many cerebral pathways from inhibitory checks.

Interactions of d-*tubocurarine and strychnine.* As may be expected from the dual action of each of these drugs, the effect produced by their combination is complicated. Since such drugs act chiefly on inhibitory synapses, weak concentrations of both produce excitatory synergism. Either drug in isolation can eventually block excitatory synapses as well as the inhibitory and, consequently, *d*-tubocurarine may block convulsant activity induced by strychnine. This duality of action becomes explicable in the face of the presence of different types of synaptic electrogenesis and of the differential sensitivity of the respective synapses to the drugs.

Other drugs. Our work on specific synaptic complexes has not yet been extended to the use of other synaptic drugs of the common type. The effects of LSD and other compounds are discussed by Purpura.[7]

SYNAPSES OF THE CEREBELLAR CORTEX

Information is absent on the intimate electrophysiology of the cerebellar neurons. Nevertheless, it seems highly likely that the phenomena are in general identical with those of spinal and cerebral cells. This conclusion is supported by an analysis of the action of some drugs on the cerebellar synapses.

Action of d-*tubocurarine.* The surface negative, and probably dendritic

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* The enormous enhancement of the surface negative response seen in FIGURE 23 when the inhibitory PSP are selectively blocked indicates that the normal response to the stimulus (*A*) is only the net predominance of depolarizing over hyperpolarizing PSP. It may be estimated that more than 80 per cent of the total synaptic activity does not appear as an electrical response because the electrogenesis of opposite signs in the total population of active synapses cancel.

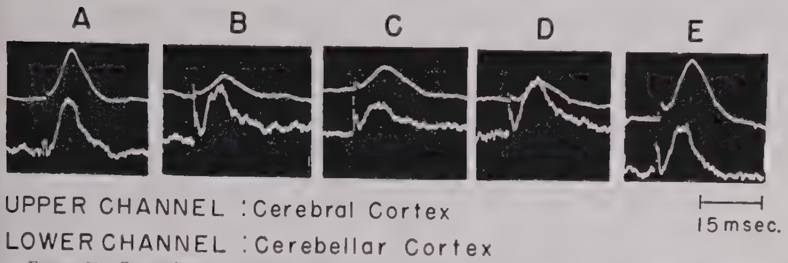

FIGURE 24. Comparisons of blockading effectiveness of *d*-tubocurarine on dendritic synapses in the cerebral cortex and the cerebellar. Upper traces, surface response from suprasylvian gyrus to a stimulus 4 mm. distant. Lower traces, from vermis, stimulus 4 mm. away, on the same folium. Simultaneous records. (*A*) Control. (*B*) Two minutes after injection of 2 mg./kg. *d*-tubocurarine. (*C* to *E*) Five minutes, 10 minutes, and 20 minutes after the injection. The cerebellar response was depressed later and more transiently than was that of the cerebral dendrites[145a].

potentials of the cerebellar cortex are also blocked by *d*-tubocurarine[145a, b] (also Purpura and Grundfest, to be published). The synaptic organization of the cerebellum, however, seems to differ markedly from that of the cerebral cortex, and these differences are reflected in their different behavior toward drugs. Some types of synaptic drive appear to be more powerful in the cerebellum than they are in the cerebrum. Thus, disappearance of dendritic potentials evoked by stimulating the cortical surface is achieved more easily with *d*-tubocurarine at the cerebral than it is at the cerebellar cortex (FIGURE 24).

Inhibitory and excitatory cerebellar synapses. A second feature of cerebellar organization is disclosed by its ability to respond at high frequencies to afferent drives, whereas the cerebral cortex cannot be so driven.[156] This behavior appears to be associated with the remarkable unresponsiveness of the cerebellar cortex to topical application of strychnine[157a, b] and curare.[154] As indicated above, strychnine appears to effectuate convulsant activity in the cerebrum by a release of the latter from many inhibitory checks. Absolute or relative absence of such inhibitory systems in the cerebellum can account for both cerebellar phenomena.*

On the other hand, metrazol functions primarily as an activator of excitatory synapses. Local application of this drug to the cerebellar cortex produces "seizure" electrocortical activity in this tissue, as it does in the cerebral.

Interactions of strychnine and d-*tubocurarine.* The specific differences in the organization and synaptic potency of cerebral and cerebellar synapses and their different sensitivities to drugs are clearly demonstrated by the different effects of drug interactions (FIGURE 25). A subconvulsant dose of strychnine followed by a larger dose of *d*-tubocurarine exerts entirely different actions in the two systems. The cerebral cortex becomes inactivated and the electrocorticogram is then flat. The previously relatively quiescent cerebellar cortex, however, develops remarkable seizure activity. This has been demonstrated to be due to a driving of the cerebellum by ascending pathways, since

* Other pharmacological experiments support this conclusion. Unlike the case in the cerebral cortex, the dendritic responses of the cerebellar cortex are not enhanced when *d*-tubocurarine is injected into a heparinized preparation. Granit and Phillips[157b] have, however, observed some inhibitory activity in Purkinje cells recorded with microelectrodes in the depth of the cerebellum. This inhibition may involve axosomatic synapses, whereas surface recordings are concerned primarily with the PSP of axodendritic synapses.

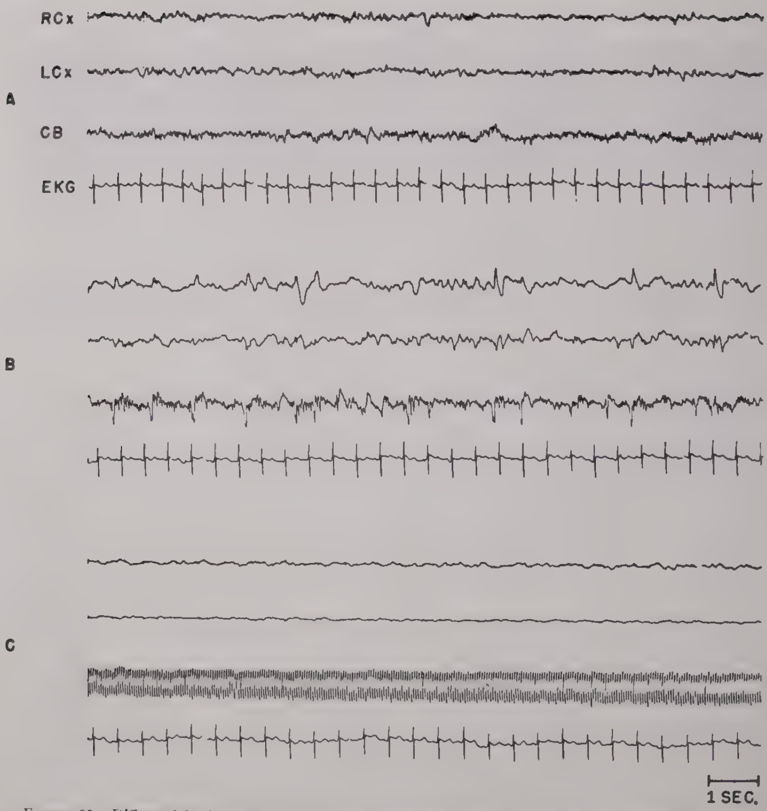

FIGURE 25. Differential effects of strychnine and *d*-tubocurarine on the cerebral and cerebellar responses. ECG: bipolar records, anterior sigmoid to anterior suprasylvian gyri of the right (RC_x) and left (LC_x) cerebral cortices. Bipolar cerebellar leads from the vermis (C_B). (*A*) Control. (*B*) Thirty minutes after 0.7 mg./kg. strychnine sulphate i.v., the tracings show the maximum, but subconvulsive activity evoked by the drug. (*C*) Five minutes after injecting 5 mg./kg. *d*-tubocurarine. The cerebral activity disappeared as a result of the latter drug. The cerebellum developed violent and rapid activity which was caused by its driving from the brainstem and spinal cord (unpublished work with D. P. Purpura).

decoupling of these from the cerebellum immediately and permanently stops the cerebellar activity. The decoupling does not affect the spinal and brainstem activity that was initiated by the combination of the subconvulsant dose of strychnine and the *d*-tubocurarine.

The analysis of these drug actions therefore is relatively simple. In the spinal cord, the *d*-tubocurarine apparently acts in synergism with the strychnine, enhancing blockade of inhibitory synaptic mechanisms and thereby releasing intense excitatory drive. The latter, in turn, drives the cerebellar synapses that have little or no inhibitory supply.

Interaction of metrazol and d-*tubocurarine.* The excitatory effects of metrazol on the cerebellum can be blocked by *d*-tubocurarine, and this action is to be expected, since the latter drug can inactivate the excitatory cerebellar synapses. As in competitive inhibition, further addition of metrazol can relieve the curare blockade and more *d*-tubocurarine can reinstitute this.

Interaction of metrazol, d-*tubocurarine, and strychnine.* This apparently complex combination is highly instructive. Cerebral quiescence and intense cerebellar activity evoked by combined strychnine and *d*-tubocurarine can be reversed by metrazol. When the cerebral cortex then becomes active the cerebellar strychnine seizure pattern disappears. Injection of more *d*-tubocurarine blocks the cerebral activity and reinstitutes that previously seen in the cerebellum. The mechanisms of this action appear to involve inhibition of spinal and brainstem activity by the cerebral cortex. When the latter is temporarily activated by the metrazol, the spinal driving of cerebellar activity is eliminated.

The foregoing examples provide specimens of the applicability of the view that central synapses, like the peripheral, can be analyzed in terms of specific electrogenic mechanisms and by the study of the effects on these of synapse-activating and -inactivating drugs. In most of the instances cited, the experiments were formulated in terms of these concepts and the results predicted beforehand. The case of the interaction of heparin and *d*-tubocurarine was unexpected only because of previous lack of knowledge of the interaction of these two drugs in peripheral synapses.

THE TRANSMITTERS OF CENTRAL SYNAPSES

General conditions. Relatively little attention has been paid thus far to the specific types of transmitters that may be involved in different synaptic systems. Chiefly, this omission stems from the general hypothesis that primary attention must be paid to the main properties of synapses, namely, that they are chemically excitable and that they may exert either inhibitory or excitatory actions that depend solely on the nature of their electrogenesis. The nature of their normal excitants is assumed to determine the relative sensitivity of a given postsynaptic component to one drug or the other. In some cases, this sensitivity may take on an absolute aspect, and this is the basis for the commonly accepted classifications of drugs. These are not superseded in the concepts developed here, but they are viewed as details sometimes of little importance to the general theory of drug action, even though their importance in therapy and in specific cases may be exceedingly great.

Putative transmitters. As to the nature of the central synaptic transmitters, the writer confesses to ignorance, referring the readers to authorities on synaptic pharmacology.[127, 158] The growing number of experiments demonstrating that central nervous activity involves a variety of neurohumors, some of long-term action[7] and, indeed, the present analysis itself, based on the existence of chemically induced synaptic transmission, should serve to stimulate a systematic search for such transmitters and for adjuvants.[159] In this connection researches on psychotogenic drugs or on tranquilizing agents can have an important impact.

One consequence of the analysis presented above, however, is the emphasis that caution must be exercised in interpreting mechanisms of drug action.

Basing itself on the general pharmacology of synaptic drugs, the work reported here attains some measure of success only to the extent that it is capable of interpreting drug effects and their interplay by paying attention to the specific anatomic and functional properties of individual synaptic systems. The analysis would appear to contraindicate, on the one hand, conclusions as to mechanism drawn from generalized, over-all effects on the whole organism or on its central nervous activity and, on the other, deductions as to central nervous mechanisms of drug action developed from systems that lack the specific complexities of the various parts of the nervous system or that may have other types of complexity not related to that of the nervous system.

Conclusions and Summary

The analysis of the synaptic action of drugs made in this paper is based on the following considerations:

(1) The synapses of the central nervous system are similar to those of the peripheral junctions with respect to their modes of excitation and inactivation; their types of electrogenesis; their differentiation from the electrically excitable, spike-generating components of the cell membrane; and the nature of the interactions between these different electrogenic effects.

(2) Synaptic membrane components, excitable chemically but not electrically, can produce either depolarizing or hyperpolarizing electrogenesis depending upon their specific molecular nature. The former is primarily excitatory to the spike-generating conductile membrane, and the latter is inhibitory. Under certain conditions, however, excitatory synaptic electrogenesis can also block the development of spikes.

(3) By its nature as a chemically but not electrically excitable system, postsynaptic membrane can develop a graded electrogenesis that can be long sustained.

(4) Also stemming from its chemically excitable nature, postsynaptic membrane of either electrogenic category is, at some synaptic sites, relatively more excitable by certain substances, and by others in other regions. The same is true for drugs that inactivate postsynaptic electrogenesis, either reversibly or irreversibly. These drugs are termed respectively synapse-activating and synapse-inactivating compounds.

(5) Other substances, some not usually suspected of synaptic action, may interact with postsynaptic membrane to enhance or to depress its sensitivity to either category of synaptic drugs.

(6) Different synaptic complexes of the central nervous system possess different relative proportions of the two general categories of synaptic membranes. Furthermore, in these complexes differences may exist with respect to the sensitivity of either or both categories (excitatory and inhibitory synapses) to the various synaptic drugs.

(7) Anatomical and physiological properties of the systems of presynaptic influx, of the synaptic complexes, and of their outflow in the electrically excitable, conductile postjunctional components also contribute to the over-all effects.

(8) Analysis of drug actions on synapses must therefore be made first, in

terms of the general properties of the different electrogenic mechanisms and second, with respect to their specific constitutions and organizations.

The data presented in this paper and in that of Purpura,[7] while they constitute only an initial survey of some of the numerous central synaptic complexes, appear to offer considerable evidence for the usefulness of the approach.*

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* This preliminary survey of synaptic pharmacology does not include references to the classical papers in which Dale and Loewi, building on the earlier work of Langley and Elliott, established the facts of chemical transmission.[70] Since writing this account, however, a deeper perusal of current literature has brought to attention earlier work in which the views incorporated in the foregoing were suggested. These are cited here. Bacq and Monnier[162] proposed that inhibitory synaptic actions were effected through hyperpolarization and excitatory through depolarization of the postjunctional cell. At the time (1935), no mechanism for this was known. Monnier[163] suggested that these electrogenic effects operated in vagal and sympathetic actions on the heart. Neuromuscular blockade and contraction of some types of muscle by acetylcholine were correctly attributed to the depolarizing action of the drug by Brown.[164, 165] In a brief discussion, the latter[166] made a number of correlations between depolarizing electrogenesis and contraction, including the actions of excitatory drugs on smooth muscle. Feldberg[167] analyzed the mechanism of neuromuscular transmission, of its blockade and of contractures in the same way as has been done in the present paper.

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Discussion of the Paper

Mary A. B. Brazier (*Massachusetts General Hospital, Boston, Mass.*): I am interested in the list of drugs that, as H. Grundfest has shown, block synaptic transmission to dendrites. It would be interesting to determine whether these same drugs influence those components of the EEG that may reasonably be assigned to axodentritically conveyed impulses from the diffuse projection system. I might add that I also had noticed the "protecting" action of heparin against the influence of *d*-tubocurarine.

Part III. Serotonin and Its Possible Role in the Nervous System

NEUROCHEMISTRY AND SEROTONIN: A CHEMICAL FUGUE

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While serving my internship at the Presbyterian Hospital in the City of New York I was summoned to hear a lecture by Richard Wilstätter, newly arrived from Munich, Germany. The "summons" came from none other than the lecturer himself, which seemed odd, to say the least. I had no claims to any sort of fame. During an interview after his lecture, he invited me to come to the Kaiser Wilhelm Institute in Munich to build a department of brain chemistry (FIGURE 1). He said he would be adviser to the department along with a retired business man, James Loeb. Why I was selected I have not yet found out. The smattering of German I had learned in school was not very practical. I could fight a dragon named Fafnir in German, but I couldn't order a glass of beer. As someone said, I knew so little and knew it so fluently. I accepted the invitation, however, and so began the first department of neurochemistry, so far as I know, in the world. The true father of neurochemistry was, of course, J. L. W. Thudichum (FIGURE 2), of whom I have already written at some length.[1]

When, disappointed by the political scene, I left Germany in 1931, I could find no opportunity in the United States to carry on further work in brain chemistry. Somewhat reluctantly, I closed my immediate career in this field by writing a book, *Chemistry of the Brain*,[2] which was published in 1937. Ordinarily, publishing a book is a fairly certain way to become an authority. But revision of the volume became necessary, and only then did I realize that I did not know enough to revise my own book and that the field had taken on new life and expanse. I called on old friends, Allen Elliott and Judah Quastel. Even they decided it essential to call in more experts. The result was *Neurochemistry—The Chemical Dynamics of Brain and Nerve*, published in 1955.

Work at the Rockefeller Institute for Medical Research, New York, N.Y., with that great and good man, D. D. Van Slyke, began in 1931 on problems of cardiovascular disease. Soon I was engrossed in an attempt to separate and identify the pressor and the vasoconstrictor from the depressor and the vasodilator substances in tissue and blood. An elusive substance believed to raise blood pressure, contained in the kidneys and called renin, caught and held my attention. It still has my attention twenty-six years later, along with one of the products of its action, angiotonin. There was some doubt, even in 1931, as to whether renin really existed. It soon became apparent that the search for it would inevitably extend from the kidneys to the blood or the serum. The serum of normal people and animals, however, contained large quantities of vasoconstrictor substances. This observation had, indeed, been made almost 100 years ago. I could find no way pharmacologically to block these vasoconstrictors to get them out of the way. For years they, or it, plagued

FIGURE 1. The Kaiser Wilhelm Institute for Psychiatry in Munich, Germany, 1928.

FIGURE 2. J. L. W. Thudichum (February 28, 1901).

me because any claim of finding substances such as renin, angiotonin or noradrenalin in blood always conflicted with the argument that the vasoconstriction elicited by extracts of blood was due to the serum vasoconstrictor. Even heparinizing the animal before blood was drawn did not solve the problem for reasons I cannot discuss here.

It seemed clear that this substance was to be a roadblock of no inconsiderable dimensions. Then came World War II, and Corcoran and I began work on shock and hemostasis. Among many others, the problem of serum vasoconstrictor again arose, this time on two counts: (1) the possibility that the vasoconstrictor was active in hemostasis, causing the blood vessels to contract around a clot forming in the vessel and so making a blood-tight seal; and (2) the question as to whether it was safe to administer serum that contained this vasoconstrictor in the treatment of shock.

I tried many biological assay methods in order to prepare the ground for chemical isolation, but most of them were not sufficiently sensitive. Finally the perfused rabbit's ear and suspended intestinal strips were chosen as at least useful, and work was begun in 1940. Six years later we were joined by Arda Green and Maurice Rapport. With their expert work it was possible finally to prepare an extract of serum from which derivatives of a highly active vasoactive material were crystallized. A good deal about the structure of this extract was learned and, in 1947, we suggested that it was closely related to tryptamine,[3-8] and we gave it the name serotonin, because of its origin in serum and its tensing action on smooth muscle. The final structure was settled by Rapport's paper[7] in 1949 showing it to be 5-hydroxytryptamine complexed with creatinine and sulfuric acid. Hamlin and Fischer[9] first synthesized the material and this was quickly followed by the reports of others who had been working independently. I was able to show that this synthetic material is pharmacologically identical with the naturally occurring vasoconstrictor.

From an entirely different point of view, V. Erspamer, in Italy, had been interested in extracts of stomach mucosa and in certain mollusks that stimulated the intestine and the uterus. He thought the active material in his extracts, which he named enteramine, was a diphenolic or polyphenolic amine but in 1952, with B. Asero, he found it to be identical with synthetic serotonin. Subsequently Erspamer has published a series of most interesting papers on the metabolic and physiological properties of serotonin, which I have reviewed elsewhere,[11] along with other earlier studies.

Since 1952, when synthetic serotonin was made available to investigators, a very large number of papers have appeared describing its varied properties. Indeed, the vascular actions alone were so varied that J. McCubbin and I felt impelled to create a new word to describe it—"amphibaric." I don't think anybody uses the term, but there it is in case of need.

What, however, has all this to do with neurochemistry? When Betty Twarog, a pupil of J. H. Welsh of Harvard University, Cambridge, Mass., joined us, it seemed worth investigating the distribution of serotonin in the mammalian body. The clam heart assay, which stems directly from Welsh's splendid work[12] on the function of serotonin in invertebrates, was used. The clam heart, along with the estrus rat uterus as used by Erspamer, is extraordinarily sensitive to serotonin and relatively insensitive to most other vasoactive agents. We found serotonin present in relatively large quantities in the brain, which understandably came as a genuine surprise. At about the same time, Amin, Crawford, and Gaddum[13] also found that the brain contained more than its share of serotonin. They subsequently prepared a beautiful map of its distribution in the brain that shows that it closely follows the distribution of noradrenalin. The highest concentrations were in tissues associated with the central autonomic representation.

At approximately the same time John Welsh was working on the effects of serotonin on the hearts of mollusks, and he made the startling observation that not only was 4 times as much serotonin as acetylcholine present in the ganglia but that agents blocking serotonin also abolished nervous excitation. From these and other observations he suggested that serotonin is an effector substance produced by nerve cells. It is, I think, widely accepted that substances other than acetylcholine may be involved in ganglion transmission in invertebrates, while the acetylcholine mechanism largely accounts for it in vertebrates. Whether serotonin or other indole derivatives play a part in vertebrates is a subject of current investigation.

A discovery was made in the research laboratory of the H. G. Sandoz Company in Basel, Switzerland, on April 16, 1943, that would have fascinated investigators and clinicians alike had they known about it at the time. That afternoon A. Hofmann (FIGURE 3) noticed a peculiar sensation of vertigo and restlessness while working in the laboratory. In a dreamlike state he left for home. There was a peculiar state of "drunkenness" characterized by exaggerated imagination. "With my eyes closed, fantastic pictures of extraordinary plasticity and intensive color seemed to surge towards me." After about 2 hours this effect passed off, and he felt quite normal.

He had been working with *d*-lysergic acid diethylamide (LSD), a substance synthesized in 1938 by Stoll and Hofmann, and he had unintentionally inhaled a minute amount of it. Investigators in this field are entirely familiar with the rest of the story.

The ability of LSD to block the action of serotonin in such small amounts is matched only by the bromine derivative of lysergic acid diethylamide (abbreviated BOL). It is curious, however, that the brom derivative has no hallucinogenic properties, in sharp contrast with the parent compound as shown by the important work of E. Rothlin in Basel. Why the substitution of 1 atom of bromine should so profoundly alter the molecule's effect on the psyche is a problem of major importance.

Regardless of the parts that future work may show LSD and BOL to play in cerebral function, their importance is established by the fact that they provided a nidus around which interest in the study of mechanisms of cerebral function formed. It is hard to see just why this was so. It had long been known that mescaline produced hallucinations, but this fact seemed to stimulate no especial interest. Neither, however, did Hofmann's work on LSD in 1943. Undoubtedly Gaddum's demonstration[14] of an antagonism between serotonin and LSD on the uterus of a rat helped, along with Woolley and Shaw's important program[15] of synthesizing competitive antiserotonins. Woolley recognized that, among these and the other antagonists of serotonin, there were substances that caused mental aberrations. Blockade of the serotonin in the brain thus seemed to be responsible for schizophrenic-like states elicited by LSD. Like most scientific problems, however, it is not that simple.

Bufotenin, the dimethyl serotonin, seems to be the active material of the

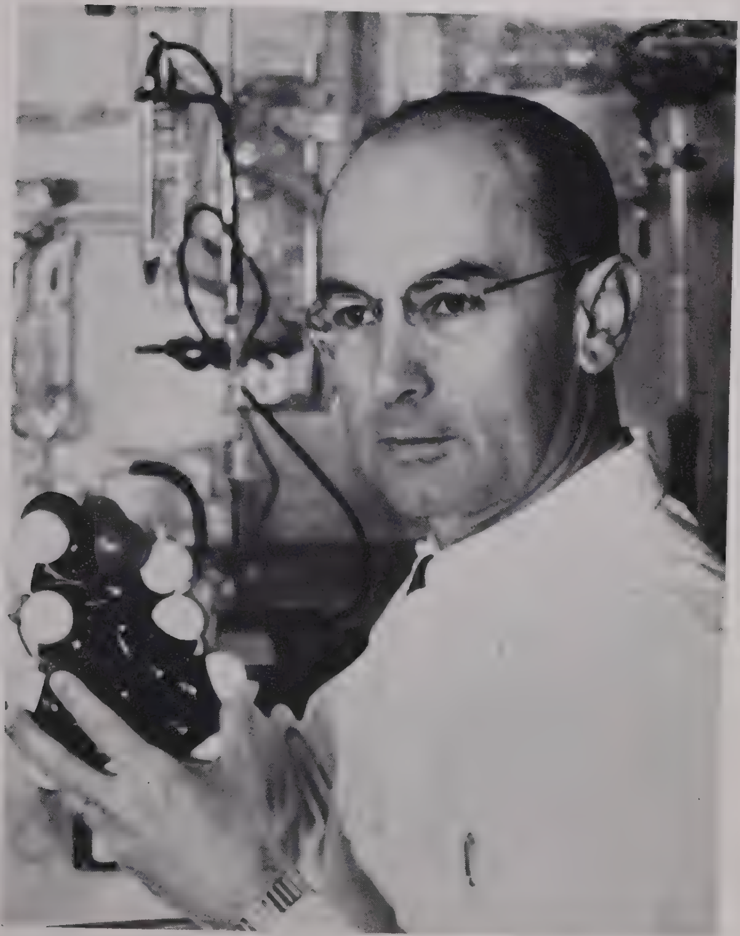


Figure 3. A. Hofmann.

Cohoba bean, used in Haiti to produce hallucinations.[16] The situation in relation to serotonin seems reversed, for now the dimethyl derivative seems to be causing the hallucinations, rather than the lack of serotonin. Perhaps there is an analogy here to the high specificity of LSD and BOL, where so slight a change as the substitution of one atom of bromine entirely destroys the ability of the molecule to produce hallucinations. It is just possible that when dealing with psychic processes the specificity of action is of a very high order, perhaps like that in immune processes. If thought is indeed associated in some way with cerebral metabolism, and if there is orderly building up and breaking down of the brain itself, as suggested by the concept of the dynamic state of the body, then it is possible to understand how disturbing substances that interfere with normal metabolism of brain cells might be. Serotonin and/or its derivatives in the broadest sense may well belong to the metabolic substances necessary for the normal functioning of the nervous system.

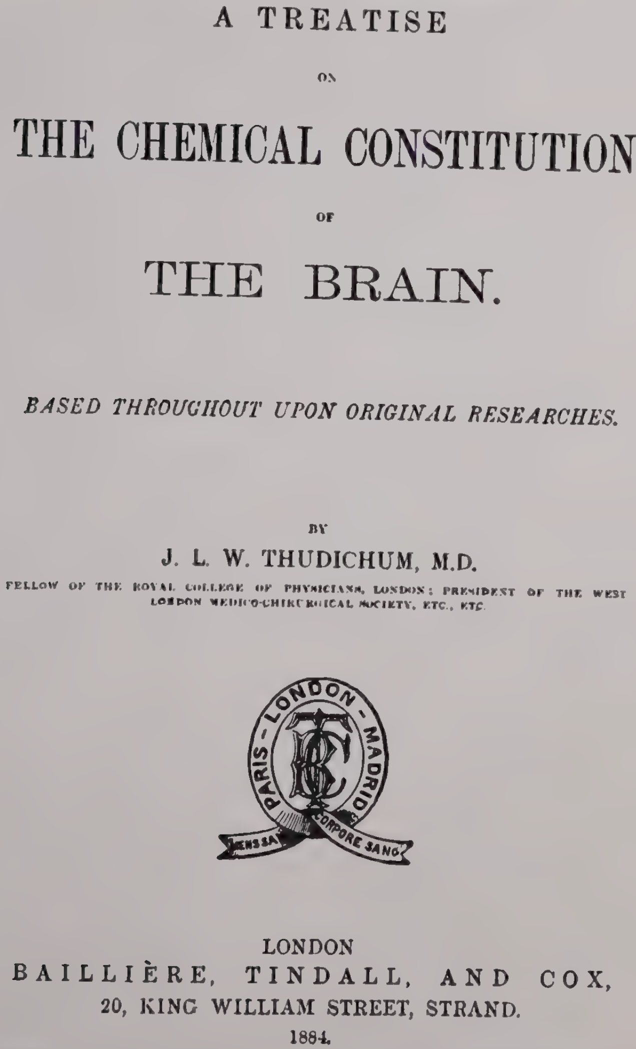
Last year Brodie and his group[17] made the interesting observation that serotonin markedly potentiates the hypnotic action of hexobarbital, and that this potentiating action is antagonized by prior administration of LSD. Further, they found that reserpine, which also potentiates hexobarbital hypnosis, causes serotonin bound by brain cells to be liberated and thus become vulnerable to destruction by monoamine oxidase. This potentiating action, too, is blocked by LSD. Although reserpine itself rapidly disappears from the brain, the brain's inability to retain serotonin persists. The serotonin which is not bound to brain cells is considered the mediator of the action of reserpine. If this mechanism proves to be the chief one responsible for the action of the so-called "tranquilizing agents" it would bring us a step closer to understanding a function of brain itself.

If LSD had not been brominated, the action of LSD on serotonin would hardly have been questioned. Cerletti and Rothlin[18] showed that the brominated product, BOL, antagonizes serotonin pharmacologically, but is not psychotomimetic. Indeed, Sollero, Salmoiraghi, and I[19, 20] as had Rothlin, found BOL more active than LSD as an antagonist on the uterus and the intestine and, further, that it blocked the prolonging effect of serotonin and reserpine on the hypnotic action of hexobarbital. Thus the mechanism of the hallucinogenic action of LSD as mediated by serotonin becomes less sure, although alternative hypotheses do not allow this mechanism to be dismissed. This is exemplified by evidence gathered by Salmoiraghi in our laboratory that the facilitory and inhibitory actions of LSD depend on dosage. Elsewhere in these pages, however, Rothlin tells us about all of this and Hoagland reviews the biochemical changes as well.

Still another highly significant contribution to the problem was made by Marrazzi and Hart,[21] who showed serotonin to be a powerful inhibitor of synaptic transmission. These investigators suggested that with generalized synaptic inhibition the over-all pattern of activity would depend on variations in synaptic thresholds throughout the nervous system. They see mental disturbance as an imbalance between adrenergic or "serotonergic" inhibition and cholinergic excitation in the more susceptible cerebral synapses.

Studies on the chemical degradation of serotonin were initiated by Witkop and Blaschko and then became largely the province of Udenfriend and his associates Titus and Weissbach[22, 23] and Erspamer.[10] Blaschko has shown that serotonin is an active substrate for monoamine oxidase. Degradation seems to depend chiefly on this enzyme. Tryptophan, as Udenfriend has demonstrated, is the precursor of serotonin. It is converted to 5-hydroxytryptophan and decarboxylated to serotonin which, in turn, is oxidatively deaminated, probably via 5-hydroxyindoleacetaldehyde, to 5-hydroxyindoleacetic acid. The latter is excreted in urine.

In a way, a quite unexpected gift came from the field of cardiovascular diseases. It was nature's own experiment in the manufacture of serotonin, and



A TREATISE

ON

THE CHEMICAL CONSTITUTION

OF

THE BRAIN.

BASED THROUGHOUT UPON ORIGINAL RESEARCHES.

BY

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LONDON
BAILLIÈRE, TINDALL, AND COX,
20, KING WILLIAM STREET, STRAND.
1884.

FIGURE 4. Title page of the first work on the chemistry of the brain, published in 1884.

it was discovered in somewhat the following way. It had been observed by Biörck, Axén, and Thorson[24] that metastatic carcinoid (argentaffinoma) was often associated with a syndrome characterized by a flushing, valvular disease of the right side of the heart, "asthma," and by diarrhea. Erspamer had suggested that serotonin might have its origin in the argentaffin enterochromaffin cells. Since carcinoid seems to originate from these cells, Lembeck[25] extracted one of these tumors and found it full of serotonin! He attributed signs and symptoms of metastasizing carcinoid to the serotonin. Udenfriend, Titus, and Weissbach[26] had developed a method for the estimation of 5-hydroxyindoleacetic acid in the urine, and Corcoran and I applied it to several patients with carcinoid. The results of their studies were combined with ours in a note published jointly in *The Lancet*.[27] Thus was first established a simple, specific method dependent on urinalysis for the diagnosis of a malignant tumor.

I shall not go further into the problem of intermediary metabolism, as Udenfriend, who has done most of the work, does this so much more understandably elsewhere in this publication. In retrospect, the carcinoid story is a curious and unanticipated one, and it illustrates the odd twists that science takes. Who would have thought that a vasoconstrictor substance forms when blood clots, and that, on teleological grounds concerned with hemostasis, it would turn out to be the important constituent of a tumor or, for that matter, an important constituent of the brain?

I hope it is now clear to you how my interest in the chemistry of the brain began 28 years ago, went into storage for several years and, finally, revived as the result of the discovery of serotonin. The search for serotonin began as a problem of cardiovascular disease, not of neurochemistry.

This sort of adventure in science strikes me as something quite different from serendipity. The story since the synthesis of serotonin illustrates the great value of making a seemingly useless substance derived from the animal body available to large numbers of investigators. Using it, the imaginations of such workers become fired by some facet of the action of the material undreamed of by the discoverers.

I think you will agree with me that neurochemistry, and now psychopharmacology, have been a long time in growing. The formal, if very inconspicuous births of these fields of knowledge should be placed in 1884 with the appearance of Thudichum's paper entitled *A Treatise on the Chemical Constitution of the Brain*. Thereafter, however, these fields lay dormant (FIGURE 4) until about 1928, when they were resurrected first in Munich, Germany, and then in England. But the growth and delineation of a field of endeavor requires, as I had reason to find out, more than merely a desire to cultivate it. What is needed is a specific discovery that points to the usefulness of something. The hallucinogens, the tranquilizers, and their metabolic partners have, I think, provided this.

A musical fugue is a composition in which a number of parts or voices combine successively in the development of a theme after its establishment by one part or voice. Should not a chemical fugue be similarly developed? This is, I think, now occurring. We are witnessing, and participating in, the rebirth of a new branch of knowledge dealing with the chemical substrates of thought, from a theme established long ago. Unless I exaggerate, a new era is indeed upon us.

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BIOCHEMICAL FINDINGS RELATING TO THE ACTION OF SEROTONIN

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The presence of serotonin in the brain was first reported by Twarog and Page[1] and by Amin, Crawford, and Gaddum,[2] who used bioassay procedures for its detection. Serotonin can also be demonstrated in the brain by using a spectrophotofluorimetric procedure that has been shown to be specific by countercurrent distribution studies and by comparison with a bioassay technique.[3] The finding of serotonin in the brain and the demonstration of the marked central effects of many indole compounds have suggested that serotonin plays an important role in the central nervous system. The present studies concerning the localization and the manner in which serotonin is made and destroyed in the brain, normally and under certain experimental conditions, lend further support to the thesis that serotonin is involved in brain function.

Localization

The serotonin in the brain is not randomly distributed, but is localized in definite areas (TABLE 1).[4] Thus the primitive portions of the brain, such as the hypothalamus and midbrain, contain relatively large amounts. The cortex and cerebellum contain very little. The enzymes, 5-hydroxytryptophan decarboxylase which makes serotonin, and monoamine oxidase which metabolizes it, are also found in the brain. Monoamine oxidase is widely distributed in the brain but, like serotonin, it is also most highly concentrated in the hypothalamus (TABLE 2).[4] According to Gaddum and Giarman,[5] the localization of 5-hydroxytryptophan decarboxylase in the brain also parallels that of serotonin. The localization of serotonin and of the enzymes that make and destroy it is asymmetric, so that the highest concentrations occur in the more primitive areas of the brain. This localization may be a clue as to where, in the central nervous system, serotonin functions.

Formation

The over-all scheme of serotonin formation and metabolism is shown in FIGURE 1. The data for this scheme were obtained from studies on mammals, toads, and bacteria.[6] Although, in the intact animal, tryptophan is converted into serotonin, it is not known in which tissue the hydroxylation occurs. As yet no evidence has been obtained indicating that the brain can hydroxylate tryptophan to yield 5-hydroxytryptophan. Whether the brain makes this intermediate or whether it is transported to the brain from a peripheral source is not known. However, it appears fairly certain that the serotonin normally found in the brain is not brought across the blood-brain barrier from peripheral sources, since relatively large amounts of peripherally administered serotonin

TABLE 1
5-HYDROXYTRYPTAMINE CONTENT OF DOG AND CAT BRAIN
Figures represent μg. per gram of fresh tissue

Species	Medulla	Pons	Cerebellum	Midbrain
Dog	0.55	0.41	0.07	0.97
Cat	0.55	0.33	0.27	1.23
	Hypothalamus	Olfactory Bulb	Cerebral Cortex	Thalamus
Dog	1.75	0.38	0.17	0.65
Cat	1.78	—	0.24	0.78

TABLE 2
RELATIVE DISTRIBUTION OF MONOAMINE OXIDASE ACTIVITY IN DOG BRAIN
Figures represent μg. of 5-hydroxytryptamine destroyed per gram of fresh tissue (homogenate) per hour

Medulla	Pons	Cerebellum	Midbrain	Hypothalamus
1250	882	970	903	3154
		Thalamus	Cerebral Cortex	
		886	844	

(60 mg./K.) were found to produce no detectable increase in the brain serotonin level in rabbits and dogs.[7] Only in mice has parenterally administered serotonin been reported to produce a slight increase in brain serotonin and then only with huge doses, 100 mg./K.[8] Further evidence that peripheral serotonin does not readily penetrate into the central nervous system comes from studies on patients with malignant carcinoid.[9] In patients with this disorder the amounts of serotonin made by the carcinoid tumors may be 100 times normal. Blood serotonin levels may be 20 times normal, and free serotonin (not bound to platelets) has been observed in blood. Normally, circulating serotonin is found exclusively in the platelets. No serotonin can be detected in the spinal fluid, however, and no central disturbance is associated with this disorder.

Although serotonin does not readily penetrate the blood-brain barrier, its amino acid precursor, 5-hydroxytryptophan, rapidly penetrates into the central nervous system where it is decarboxylated to serotonin.[10] In experimental animals, levels of serotonin as high as 10 to 20 times normal have been obtained by administering 5-hydroxytryptophan. At these high levels of brain serotonin, the animals show tremors and marked excitement. The effects observed n dogs are summarized in TABLE 3. Most of them, which have also been observed in mice, rats, rabbits and cats, are almost identical with those observed after administration of lysergic acid diethylamide (LSD).[11] Similar effects have been observed by Brodie *et al.*[12] after administration of reserpine in the presence of the monoamine oxidase inhibitor, iproniazid (isopropylisonicotinyl hydra-

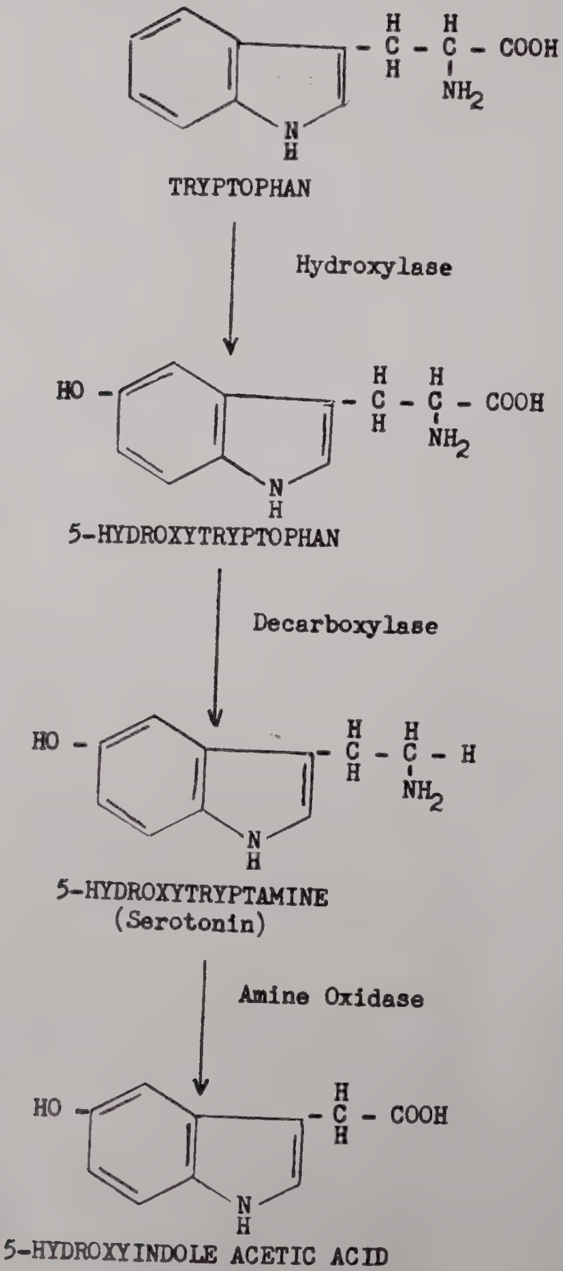
TRYPTOPHAN
Hydroxylase
5-HYDROXYTRYPTOPHAN
Decarboxylase
5-HYDROXYTRYPTAMINE
(Serotonin)
Amine Oxidase
5-HYDROXYINDOLE ACETIC ACID

FIGURE 1

TABLE 3
SOME EFFECTS OF 5-HYDROXYTRYPTOPHAN IN THE DOG*

Generalized skeletal muscle tremors
Loss of placing reactions
Postural incoordination
Lacrimation
Salivation
Piloerection
Increased heart rate
Increased gastrointestinal activity
Loss of response to visual stimuli
Pupillary dilation
Loss of the light reflex

* Sixty mg. per kg. of 5-hydroxy-*D-L*-tryptophan were administered intravenously.

ide). In the latter experiment the effects have been explained on the basis f an excess of unbound serotonin.

That the central effects of 5-hydroxytryptophan result from its conversion o serotonin can be shown by pretreating animals with iproniazid, which in- ibits monoamine oxidase. When this is done the pharmacologic effects of -hydroxytryptophan and the brain levels of serotonin are markedly enhanced ith no appreciable change in the levels of 5-hydroxytryptophan (TABLE 4).

Although 5-hydroxytryptophan can readily penetrate into the central nerv- us system, it is not possible to say whether the brain normally is supplied with -hydroxytryptophan from peripheral sources or whether it makes it itself. 'he ability of administered 5-hydroxytryptophan to elevate brain serotonin, owever, makes it a useful pharmacological tool. With 5-hydroxytryptophan : is now possible to determine the effects of excess serotonin in the brain and o study its interaction with agents such as reserpine, chlorpromazine, and SD.

Since the conversion of 5-hydroxytryptophan to serotonin is catalyzed by he enzyme 5-hydroxytryptophan decarboxylase, which requires as a coenzyme yridoxal phosphate,[13, 14] it was of interest to investigate the effects of pyri- oxine deficiency on the tissue levels of serotonin. In pyridoxine-deficient hickens it was found that the serotonin levels in brain were only 30 per cent

TABLE 4
EFFECTS OF IPRONIAZID ON SEROTONIN LEVELS AFTER ADMINISTERING 5-HYDROXYTRYPTOPHAN

Experiment	Serotonin		5-Hydroxytryptophan	
	Brain	Liver	Brain	Liver
	μg./gm.		μg./gm.	
ontrol	0.63	0.3	0	0
- Iproniazid	1.1	0.5	0	0
- 5-Hydroxytryptophan	1.3	3.7	11.8	56.3
roniazid + 5-hydroxytryptophan	4.1	6.3	15.7	58.6

Rats were given 75 mg./kg. of iproniazid, intraperitoneally. After 1 hour 5-hydroxy-*D*- -tryptophan (150 mg./kg.) was administered. The animals were sacrificed 3 hours after lministration of the 5-hydroxytryptophan.

TABLE 5

TISSUE LEVELS OF SEROTONIN IN PYRIDOXINE-DEFICIENT CHICKENS

Tissue	Serotonin μg./gm.
Brain (normal)	1.13 (7)
Brain (deficient)	0.39 (4)
Intestine (normal)	5.00 (5)
Intestine (deficient)	1.19 (6)
Blood (normal)	5.5 (3)
Blood (deficient)	0.4 (3)

Chickens were fed from hatching on a diet containing about 1 per cent of the optima quantities of pyridoxine.[21] Their food consumption was similar to that of the controls. Th values shown for each experiment are averages, the numbers in parentheses indicating th number of animals employed.

of normal (TABLE 5). The amounts of serotonin in the blood and other tissue were even more markedly reduced. Thus the neurological disturbances observed in pyridoxine deficiency may result, in part, from a deficiency in serotonin. Since, however, formation of epinephrine, norepinephrine, and histamine also involve pyridoxal phosphate decarboxylases, it is likely that th neurological disturbances in this vitamin deficiency are due to more than deficiency of serotonin.

Metabolism

Serotonin, when administered parenterally to animals, is rapidly metabolize and excreted as 5-hydroxyindoleacetic acid.[15] It has been shown that th tissue catalyst responsible for this metabolism is the enzyme known as monoamine oxidase, and that this enzyme is present in the brain.[16] It may be that monoamine oxidase regulates the action of serotonin in the same way that cholinesterase regulates the action of acetylcholine.

The properties of monoamine oxidase have been studied by Zeller and his collaborators.[17] These investigators have reported that iproniazid is a potent inhibitor of this enzyme.[18] Studies with iproniazid *in vivo* in rats and rabbits indicate that it can produce a relatively rapid and large (threefold) rise in brain serotonin. The effects of iproniazid on serotonin can also be demonstrated after administration of 5-hydroxytryptophan. By blocking the destruction of serotonin more of the amine is found after administration of a given dose of 5-hydroxytryptophan, and the intensity of the pharmacological effects parallels the level of serotonin in the brain. It is of interest that the most striking effects of iproniazid on either endogenous serotonin or after 5-hydroxytryptophan administration are observed in the brain.

The value of a monoamine oxidase inhibitor as a tool in investigations on serotonin is obvious. Although iproniazid has been useful in many studies on serotonin, it leaves much to be desired. In addition to blocking amine metabolism it is known to interact with pyridine nucleotides[19] and, like isoniazid, to cause depletion of pyridoxine,[20] making its *in vivo* actions difficult to interpret, especially in chronic studies. Although effects of iproniazid on serotonin can be readily demonstrated *in vivo* it has been found that inhibition of mono-

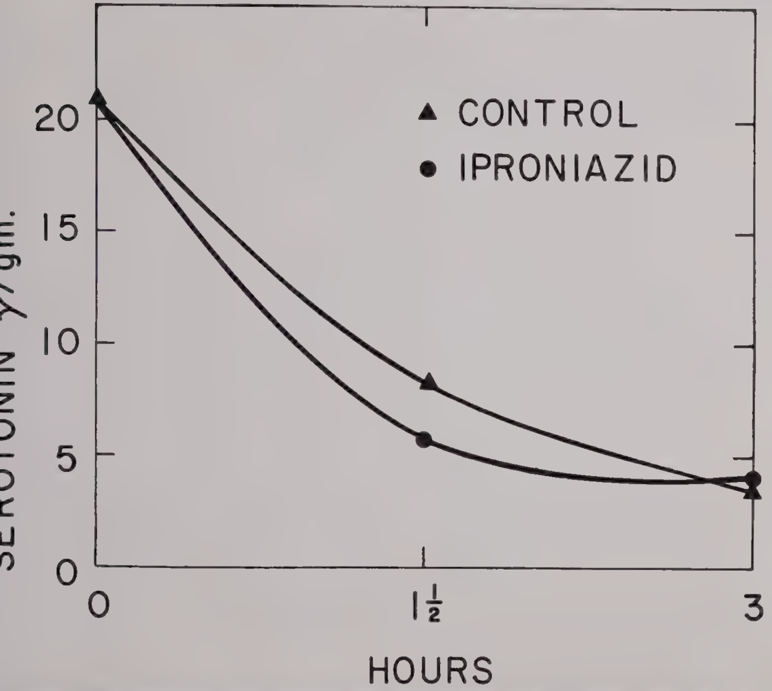

FIGURE 2. Disappearance of injected serotonin from the whole mouse, following injection, intraperitoneally, 1 mg. of serotonin into white mice (about 40 gm.). At the times specified each mouse with its urine and feces as homogenized in 0.1 N HCl and made up to volume for analysis. Each point represents several animals.

mine oxidase by iproniazid is not very great, as measured by the rate of disap- earance of administered serotonin from mice *in vivo* (FIGURE 2). It has also een found that when slices of tissue from animals pretreated with iproniazid re used, little inhibition of serotonin metabolism is found. After homogena- on of a portion of the same tissue, however, inhibition is complete. Accord- igly, although iproniazid is potentially a very potent inhibitor and has de- ionstrable activity *in vivo*, it is not too potent an inhibitor on intact cells ther *in vivo* or in slices of tissue. It would be desirable to find a monoamine xidase inhibitor more active than iproniazid *in vivo*, and without its side effects.

Turnover of Brain Serotonin

Although it is possible to label brain serotonin by administering C^{14}-labeled recursors such as tryptophan or 5-hydroxytryptophan, the total amount of erotonin in the brain of experimental animals is so small that even when high pecific activities are obtained the total counts are very low. The half life of erotonin in the stomach and the intestine, determined after administration C^{14}-labeled tryptophan, is about 10 hours.[22] Preliminary radioactive studies on brain serotonin using C^{14} 5-hydroxytryptophan indicate that its half lif is no longer than 10 hours. The rapid increase of serotonin in the brain i animals pretreated with iproniazid suggests that serotonin in the brain ma turn over even more rapidly, with a possible half life of 1 to 3 hours.

Acknowledgment

We thank George M. Briggs for making available the pyridoxine-deficien chickens.

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ROLE OF BRAIN SEROTONIN IN RESERPINE ACTION

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The discovery that serotonin occurs in the brain[1, 2] has stimulated considerable thought concerning its role in the central nervous system.[3, 4] Speculation n this direction received dramatic impetus when Gaddum[5] found lysergic acid diethylamide (LSD) to be a potent antagonist of serotonin on smooth muscle *in vitro*. This observation led to the hypothesis that the hallucinogenic agent produces its aberrant mental effects by interfering with a normal function of serotonin in the brain. Evidence in favor of the concept that serotonin is nvolved in certain aspects of brain function has stemmed from findings summarized in this paper that indicate that the central actions of reserpine are mediated through free serotonin in the brain.

Serotonin in brain tissue is normally present largely in a bound form, and is thus protected from the action of the enzyme monoamine oxidase. The amine has an asymmetric distribution: the higher concentrations are found in the brainstem, especially the hypothalamus; a lower concentration in the cortex, with virtually none in the cerebellum.[2] It is in the regions of the brain, where serotonin levels are highest, that reserpine is said to exert its central actions.[6, 7]

The central actions of administered serotonin are difficult to evaluate because t passes into the brain with difficulty, and large doses of the compound usually exert potent effects on the cardiovascular system. Fortunately, serotonin is relatively innocuous to mice, and it is possible to observe central effects in these animals after the parenteral administration of large doses of the amine. Small but measurable amounts of serotonin enter the brain. Thus 15 minutes after he intraperitoneal injection of serotonin (100 mg. per kg.) the brain level had risen from the normal value of 0.9 μg. per gm. to a value of 1.2 μg. per gm., an increase of about 35 per cent. The increase in brain serotonin was far greater than could be accounted for by the presence of blood in the brain. Even when completely freed of blood by perfusion with saline, brain tissue still had a level of serotonin about 25 per cent greater than the normal value.

Mice given 20 mg. per kg. of serotonin intraperitoneally showed central effects similar to those induced by reserpine, including a general depression of motor activity, sedation, and potentiation of the hypnotic action of barbiturates and alcohol.[8] The action of alcohol was particularly enhanced, the usual hypnotic dose being almost invariably lethal to mice pretreated with serotonin. f the mice were first given 10 mg. per kg. of lysergic acid diethylamide, however, the sedation and the potentiation of hypnotics were markedly diminished. These experiments demonstrated for the first time that LSD could antagonize a central action of serotonin in the intact animal. An objection might be raised that the dose of LSD was very large compared to that producing aberrant effects in man, but the large dose is understandable in view of the extraordinarily rapid metabolic transformation of LSD in mice (a half life of about 7 minutes).[9]

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If the central actions of reserpine are mediated through serotonin, these should also be blocked by LSD. This was found to be true. Pretreatment of mice with LSD blocked not only the marked potentiating effects of reserpine on barbiturates and alcohol[10] but overcame its depressant effects. Because of its rapid biotransformation it had only a transient analeptic effect, and the mice rapidly reverted to a somnolent state.

An interaction between reserpine and serotonin was shown more directly by giving reserpine (5 mg. per kg.) to dogs.[10] A marked increase, lasting about 12 hours, occurred in the rate of urinary excretion of 5-hydroxyindoleacetic acid, the major metabolite of serotonin. This observation suggested that bound serotonin in the body had been released and metabolized. A second dose of reserpine administered 24 hours after the first failed to cause another rise in urinary 5-hydroxyindoleacetic acid, an indication that the serotonin stores had been depleted by the first dose of alkaloid and had not yet been replenished.

The serotonin depots depleted by reserpine were identified by direct analysis of the intestines, blood platelets, and brains of rabbits given reserpine. After 5 mg. per kg. of reserpine, serotonin from the small intestine and platelets was liberated continuously for a period of about 16 hours, when it reached a concentration of about 10 per cent of the normal value. The concentration remained at this low level for about 30 hours and then gradually rose, reaching the original level in 5 to 7 days.[11, 12]

Brain serotonin is especially sensitive to reserpine. Serotonin was released from this tissue much more rapidly than from intestines and blood platelets. A measurable decline in serotonin levels in brain occurred within 10 minutes after reserpine administration, and within 30 minutes it had declined by about 80 per cent.[13] The maximal decline, about 90 per cent, occurred within 4 hours (FIGURE 1). The sensitivity of brain serotonin to reserpine was also apparent from the low dose, 0.1 mg. per kg., that measurably lowered the serotonin content.

Further evidence that the central actions of reserpine are mediated through serotonin came from a comparison of the serotonin-releasing activities of a number of centrally acting drugs, including various *Rauwolfia* alkaloids, chlorpromazine, LSD, morphine, barbiturates, and amphetamine.[14] The ability to release serotonin from the brain was limited to *Rauwolfia* alkaloids and, of these, to the alkaloids that exert tranquilizing effects (TABLE 1). Of special interest was the observation that isoreserpine, a pharmacologically inactive stereoisomer of reserpine, and isoraunescine, an inactive isomer of raunescine, had no effect on brain serotonin.

Additional support for the thesis that reserpine acts through serotonin came from experiments demonstrating that the pharmacological effects of the drug and the change in brain serotonin persisted long after reserpine had disappeared from brain tissue.[15] After administration of reserpine to rabbits intravenously, the drug rapidly entered the brain and achieved its maximal concentration in

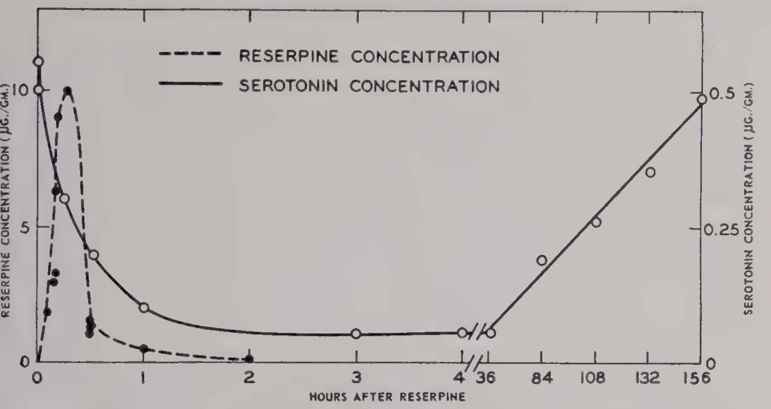

Figure 1. Concentration of reserpine and serotonin in the brain of a rabbit at various times after intra-enous administration of reserpine (5 mg./kg.). Each point represents a single rabbit.

bout 10 minutes, a time when the pharmacological effects of the alkaloid were st apparent (figure 1). The levels in brain then declined rapidly and, in bout 2 to 4 hours, reserpine could no longer be detected. While the brain evels of reserpine were declining the pharmacological effects progressively in-reased, becoming maximal in 2 to 4 hours. The low levels of serotonin and arious pharmacologic effects, including sedation, miosis, hypothermia, and ypotension, persisted for about 2 days.[16] The level of serotonin then in-reased, and the pharmacologic effects diminished. Thus certain central ac-ons of reserpine are related in time to the change in concentration of brain erotonin and not to the concentration of reserpine.

Table 1

Serotonin Concentration in the Brain of Rabbits 4 Hours after Intravenous Administration of Various *Rauwolfia* Alkaloids.

Alkaloid	Dose, mg./kg.	Sedative action	Serotonin concentration µg./gm.
one	—	—	0.57
eserpine	2	Active	0.06
eserpidine (recanescine)	2	Active	0.09
escinnamine	2	Active	0.10
ethyl reserpate syringate	3	Active	0.06
aunescine	5	Active	0.07
ethyl reserpate acetoacetate	3	Slightly active	0.34
soreserpine	2	Inactive	0.48
soraunescine	5	Inactive	0.47
ethyl reserpate	2	Inactive	0.44
eserpic acid	2	Inactive	0.52
eserpinine	2	Inactive	0·49
erpentine	2	Inactive	0.45
jmaline	2	Inactive	0.44
jmalicine	2	Inactive	0.55

It could still be argued that the change in brain serotonin was not directly involved in the effects of reserpine, but that the drug acted through a persistent metabolic product not measured by the analytical method. This possibility is unlikely, however, for several reasons. First, one of the metabolic pathways of reserpine *in vivo* is hydrolysis to methyl reserpate and trimethoxybenzoic acid. Neither of these products exerted appreciable serotonin-releasing or sedative action. Furthermore, brain serotonin declined to the same extent whether 1 or 5 mg. of reserpine was administered to rabbits. The same intensity and duration of pharmacological effects and the same time for the restoration of serotonin levels were seen with the 2 different doses (FIGURE 2). This makes it unlikely that a metabolic product was responsible for the persistent action of reserpine, as the larger dose of reserpine should have resulted in the formation of more of the metabolite than the smaller dose and therefore should have caused a more prolonged response.

Evidence that the intact reserpine molecule can release serotonin was obtained from studies *in vitro* showing that the alkaloid released serotonin from a suspension of rabbit blood platelets in plasma.[17] Under these conditions reserpine was not metabolized. A concentration of the alkaloid as low as 0.05 μg. per ml. of plasma liberated measurable amounts of serotonin while a concentration of 0.3 μg. per ml. caused a maximal rate of release. Calculation

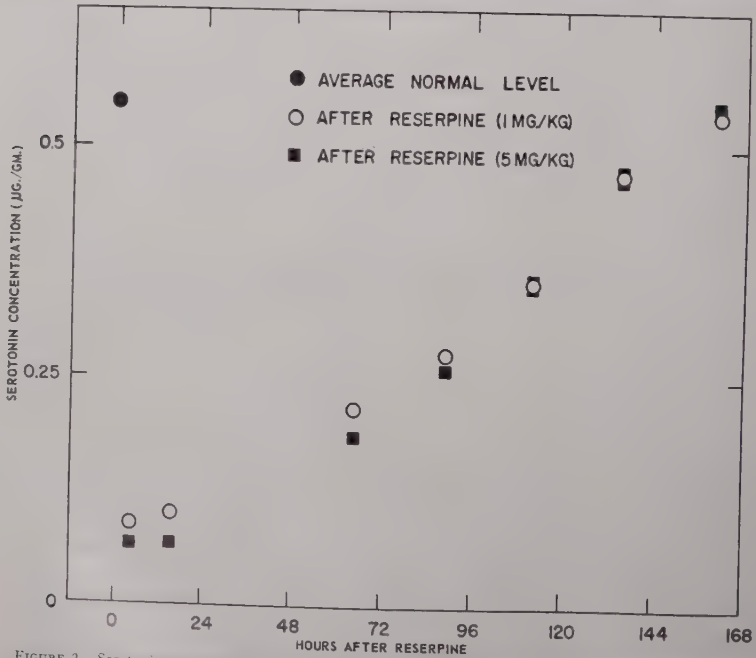

FIGURE 2. Serotonin content of the brain of a rabbit at various times after intravenous administration of reserpine (1 or 5 mg./kg.). Each point represents a single rabbit.

howed that 1 molecule of reserpine effected the release of hundreds of serotonin olecules, an indication that the phenomenon was not one of simple displacement. As in the whole animal, ability to release serotonin *in vitro* was limited o *Rauwolfia* alkaloids possessing sedative properties.

From the foregoing, it would seem that the pharmacological effects of reserpine re not merely concurrent with the change in brain serotonin, but are a consequence of it. Further studies have indicated that the primary action of reserpine is to impair the capacity of cells to bind serotonin. A comparison was ade of the amount of added serotonin taken up *in vitro* by blood platelets rom normal guinea pigs and from animals that had received reserpine 16 to 24 ours previously.[18] The platelets from the reserpine-treated animals exhibited marked deficiency in their capacity to take up serotonin.

Similar results were obtained *in vivo* for the brain. The precursor of serotonin, 5-hydroxytryptophan, has been shown by Udenfriend *et al.*[19] readily to nter the brain, where it is decarboxylated to yield serotonin. One hour after iving 50 mg. per kg. of the amino acid intravenously to rabbits, brain serotonin ose from a level of 0.5 to 1.7 μg. per gm., a net increase of 1.2 μg. Conversely, f the rabbits had been given 5 mg. per kg. of reserpine 16 hours previously, he levels of serotonin rose from about 0.05 to 0.35 μg. per gm., an increase of nly 0.3 μg. per gm. Thus, at a time when reserpine was no longer detectable n the brain, the capacity of cells to take up serotonin was still demonstrably mpaired. The possibility that reserpine had blocked the action of 5-hydroxytryptophan decarboxylase, the enzyme that catalyzes the decarboxylation of -hydroxytryptophan to serotonin, was excluded when it was shown that the ctivity of this enzyme in the brain was unchanged.

Thus it appears that reserpine acts as follows: it rapidly enters the brain and, n some unknown way, irreversibly affects the serotonin binding sites and then isappears. As a result, serotonin is released and the unstable free form is etabolized by the action of monoamine oxidase. Serotonin continues to be ynthesized in the body, and the low serotonin level that persists presumably epresents a balance between its rate of synthesis and its rate of metabolic ransformation. The effect of reserpine persists until the binding sites regain heir binding capacity or until new sites are formed.

The question that now arises is whether the pharmacological manifestations f reserpine may be attributed to the decrease in bound serotonin in the brain or o the presence of a persistent low concentration of free serotonin. The evidence is more in accord with the concept that the persistent presence of free erotonin accounts for reserpine action. In all experiments in which serotonin s used as a pharmacological agent, only the free form seems to be active. For xample, as mentioned in the first part of this paper, the sedative and potentiating effects of reserpine were mimicked in mice shortly after a parenteral injection of serotonin. The mice, however, seemed to have recovered in 90 inutes, even though the brain level of serotonin was still considerably above ormal value. This increase presumably represents bound and therefore harmacologically inactive serotonin.

Again, after injection of 5-hydroxytryptophan (100 mg. per kg.) into rats, he serotonin content of the uterus was found to be about 2.5 μg. per gm.

This tissue showed no activity if suspended in Locke's solution, but whe serotonin was added to the bath in a concentration of only 0.01 μg. per ml there resulted a strong contraction.[20] It is evident that the small concentratio of added free serotonin was active, whereas the large amount of the boun amine in tissue was inactive.

If the change in the concentration of bound serotonin were the determinin factor in the action of reserpine, one would expect that changes in the level c bound serotonin in brain would parallel pharmacological effects. This is nc the case. Thus, rabbits were heavily sedated 30 minutes after reserpine ac ministration, when the serotonin level in brain had declined to about 0.2 μg per gm. of tissue (FIGURE 1). Yet no sedation or other pharmacological effect were apparent in 72 hours, although the serotonin level had returned to th same line, about 0.2 μg. per gm. This apparent paradox can be explained b assuming that free serotonin is the cause of sedation. In 30 minutes th serotonin was still being released, and therefore a certain amount of free serotonin was present. In 72 hours, although the total serotonin level was agai 0.2 μg. per gm., a negligible amount of free serotonin was probably present since the serotonin-binding sites had recovered sufficiently to take up the amin as it was synthesized.

Recently, Cronheim has presented further evidence that free serotonin ca induce a reserpinelike action.[21] He reported that serotonin, infused intravenously into dogs pretreated with reserpine, caused a profound drop in bloo pressure. No such effect was noted in a normal dog. This result is bes interpreted by assuming that a small amount of free serotonin had passed into the brain where it could not readily be bound, thus adding to the effect of the free serotonin already present as a result of reserpine action.

Most of the observations of the effects of reserpine on serotonin depots have been made in animals following administration of relatively large doses of reserpine. It is of interest that a profound effect on blood-platelet serotonin may be observed in man after daily parenteral administration of 1 mg. of reserpine.[22] This dose in a single administration usually induced little pharmacological effect, and a varying change in platelet serotonin, but after a few days blood serotonin levels decreased by 95 per cent or more. On stopping the drug, 2 or 3 weeks were required for serotonin levels to return to normal. The cumulative effect of small daily doses of reserpine was also observed in guinea pigs. A single intraperitoneal injection of 15 μg. per kg. produced little change in brain serotonin, and a more marked change in platelet serotonin, but the daily administration of this dose for a period of 1 week reduced serotonin in blood by about 95 per cent and, in the brain, by about 20 per cent. Serotonin levels in the intestine were not significantly altered by these small doses.

Although a single small dose of reserpine causes little pharmacological effect or alteration in serotonin, the daily administration of small doses seems to exert a cumulative effect on serotonin levels, presumably by destroying a certain number of binding sites each day and, finally, it produces a definite pharmacological response. These results explain why, in the use of the drug clinically, results are not seen for several days after starting administration of the drug but may persist for a considerable time after discontinuing it.

Thus from the data assembled in this paper it would appear that the primary action of the active *Rauwolfia* alkaloids is to impair serotonin-binding sites in he body. As a consequence the serotonin in various depots including brain s released and metabolized by the action of monoamine oxidase. The serotonin that continues to be made presents a persistent low concentration of free serotonin to brain tissue. It is this free serotonin that is considered to exert he actions attributed to reserpine. These actions persist until the binding sites have recovered or until new ones are formed.

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Discussion of the Paper

Amedeo S. Marrazzi (*Veterans Administration Hospital, Pittsburgh, Pa.*): It vould seem equally plausible to explain the action of reserpine in causing serotonin to spill out of the brain of the rabbit on the basis that reserpine, which s the only one of the tranquilizers with an indolelike structure, prevents the inding of serotonin at the receptor as well as, possibly, at other sites, and that his accounts, at least in part, for the liberated serotonin leaking out of the rain. The persistent action after disappearance of the detectable amounts f reserpine in the brain could be due either to the "denaturation" of receptor ubstance or its persistent binding by small amounts of reserpine, or some reakdown product of it, that thereby becomes undetectable because it is ound and unavailable for analysis.

N. J. Giarman (*Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.*): Concerning the distribution of the enzyme that forms serotonin in the brain we have found that in many areas the presen of this enzyme corresponds well with the level of endogenous serotonin in the areas. This was found to be true in those regions where both values are rel tively high, as in various parts of the medulla, in the caudate nucleus, and i the hypothalamus. In addition, the correlation also held in those areas whe both endogenous serotonin and the serotonin-forming activity are low or no existent, as in the cerebellum and in the cerebral cortex.

Truly anomalous situations were found in the sympathetic ganglia and i the area postrema. In the former there is a high degree of serotonin-synthesi ing capacity, but neither Paasonen nor Gaddum and his co-workers have bee able to detect serotonin in this tissue. On the other hand, the area postrem contains relatively high levels of serotonin, but no enzyme for synthesizing i

Ernest Sachs, Jr. (*Hitchcock Clinic, Hanover, N. H.*): Have Shore and hi colleagues studied serotonin in the brain or spinal fluid of patients with hea injuries, brain tumors, or other neurological conditions?

Shore: We have not.

Sachs: We have been doing this for the last year in the course of studie on acetylcholine in the spinal fluid of patients with head injuries and brai tumors, and we have incidentally encountered serotonin, which normally doe not occur free in the spinal fluid.

Georg E. Cronheim (*Riker Laboratories, Inc., Los Angeles, Calif.*): Th experimental results presented by Shore, together with data on cardiovascula effects of reserpine and serotonin[1] can be explained in a somewhat differen manner.

It is postulated that two or more types of functional centers in the centra nervous system utilize serotonin. One group existing at subcortical level has cardiovascular and motor functions, and its activation by serotonin evoke hypotension, bradycardia, and "sedation." Another group, less clearly defined may be located at cortical levels and may be responsible for behavioral characteristics, or it may simply serve as a storage function (depot) to be called upon during a period of stress. Serotonin is formed at a constant rate from tryptophan and 5-hydroxytryptophan, and it is supplied to the different functional centers (receptor sites) by separate pathways (transfer mechanisms).

It is proposed further that reserpine acts by blocking selectively the entrance of serotonin into the depot. Since serotonin continues to be formed at an unchanged rate, the blockade of one pathway shifts a greater amount of serotonin via the other pathway to the subcortical centers with a resultant increased activation of their normal hypotensive, bradycardic, and sedative activity. Administration of serotonin as a slow infusion increases the amounts in the central nervous system. In untreated animals, this increase, unless extremely great, passes mainly through the depot and does not change the activity of the various centers. If the concentration of serotonin is greatly increased by injecting 5-hydroxytryptophan, this resultant excess in the "higher" centers leads to hallucinatory effects. If, however, pretreatment with reserpine has blocked the pathway to the depot, all serotonin must go to the subcortical centers, thus causing an observed additional fall in blood pressure and heart rate, and also progressive sedative effects.

This explanation classifies reserpine as an antimetabolite of serotonin, but with certain restrictions as to the place of the antimetabolite effect. The possibility of an antimetabolite relationship between reserpine and serotonin had been suggested by D. W. Woolley and also by J. H. Gaddum. The present concept, however, leads to different conclusions in that the cardiovascular and sedative effects of reserpine are not due to an inhibition or replacement of serotonin but, on the contrary, to an increase of serotonin in certain subcortical centers.

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Jurg A. Schneider (*CIBA Pharmaceutical Products, Inc., Summit, N. J.*): Evidence obtained in white mice receiving high doses (20 mg./kg., administered subcutaneously) of serotonin creatinine sulfate does not support the theory that 5-hydroxytryptamine may cause convulsions. On the contrary, serotonin given in these doses proved to elevate the threshold to electroshock considerably. It also prolonged the latency and protected 50 per cent of the mice completely against supramaximal electroshock.

Recent investigations in our laboratory indicate that some of the pharmacological effects of high doses of serotonin are opposite to those of reserpine. Thus serotonin causes a dilatation of the pupils in doses of 20 mg./kg. in mice, whereas reserpine causes a constriction of the pupil. I have mentioned the anticonvulsant effect of serotonin against electroshock. Reserpine, on the other hand, is known to lower the threshold to electroshock. Methylphenidate (Ritalin™), a central stimulant, recently has been shown to be rather specific in antagonizing the effects of reserpine. Methylphenidate, however, potentiates the tranquilizing effect of serotonin in mice. Finally, emesis in pigeons, which can be induced with reserpine at relatively small doses, can be antagonized effectively by the administration of repeated intramuscular doses of serotonin. I should like to ask Doctor Shore how these observations fit into his theory of the action of reserpine?

Shore: It might be too much to expect parenterally administered serotonin to duplicate every effect of reserpine. After injection, serotonin undoubtedly reaches many sites in the body that do not normally contain or utilize this substance.

SERIOTONIN AS A POSSIBLE NEUROHUMORAL AGENT: EVIDENCE OBTAINED IN LOWER ANIMALS*

By John H. Welsh

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Indolealkylamines are found in a wide variety of tissues and species. V Erspamer, who has made the most extensive survey of the distribution of these compounds, has summarized his own findings and those of others in 2 review (1954a and b). Erspamer's first interest was in the enterochromaffin system of the gastrointestinal tract. From the gastrointestinal mucosa of representatives of all 5 classes of vertebrates, and from the tunicates, he isolated a substance that he called enteramine, later identified as 5-hydroxytryptamine (5-HT, serotonin). He also found 5-HT, along with a series of related compounds, in the skin of a wide variety of amphibia. In the invertebrates large amounts of 5-HT were found by Erspamer in such places as the posterior salivary glands of certain cephalopods (for example, *Octopus vulgaris*) and in the hypobranchial body of a family of marine mollusks, the Muricidae. Here, the 5-HT may be a component of a venom produced by these glands.

In the past 2 years 5-HT has been found in other places among the invertebrates, as in the venom of a wasp (Jaques and Schachter, 1954) and in tentacles of certain coelenterates (Welsh, 1956). In the latter situation it may be a component of the toxin contained in the stinging nematocysts. It would appear that 5-HT has a variety of roles, of which one may be that of a substance useful in defense and in capturing or relaxing prey.

The observation of Erspamer and Ghiretti (1951) that extracts of posterior salivary glands of *Octopus vulgaris* and hypobranchial glands of *Murex* had a powerful excitor action on molluscan hearts led to the finding that 5-HT has an excitor action on the heart of the mollusk, *Venus mercenaria* (Welsh, 1953a). Extracts of ganglia of this species were found to contain a substance which is probably 5-HT (Welsh, 1953b).

It now appears that 5-HT is a constituent of the nervous systems of a variety of molluscs and that it has a neurohumoral role in this group. This paper will discuss some of the evidence for this that has not previously been reported in detail. A neurohumoral role for 5-HT is indicated in the Crustacea (Florey and Florey, 1954), and their work will be discussed along with some results of our own. Elsewhere among animals other than mammals the author is unaware of work that implicates 5-HT as a neurohumor.

Evidence from the Molluscan Heart

The heart of *Venus mercenaria*, a clam, is innervated by a pair of cardioregulator nerves arising in the visceral ganglion. When this ganglion is electrically stimulated the amplitude of heart beat is reduced or the heart stops beating. This fact has long been known. Sometimes during recovery there

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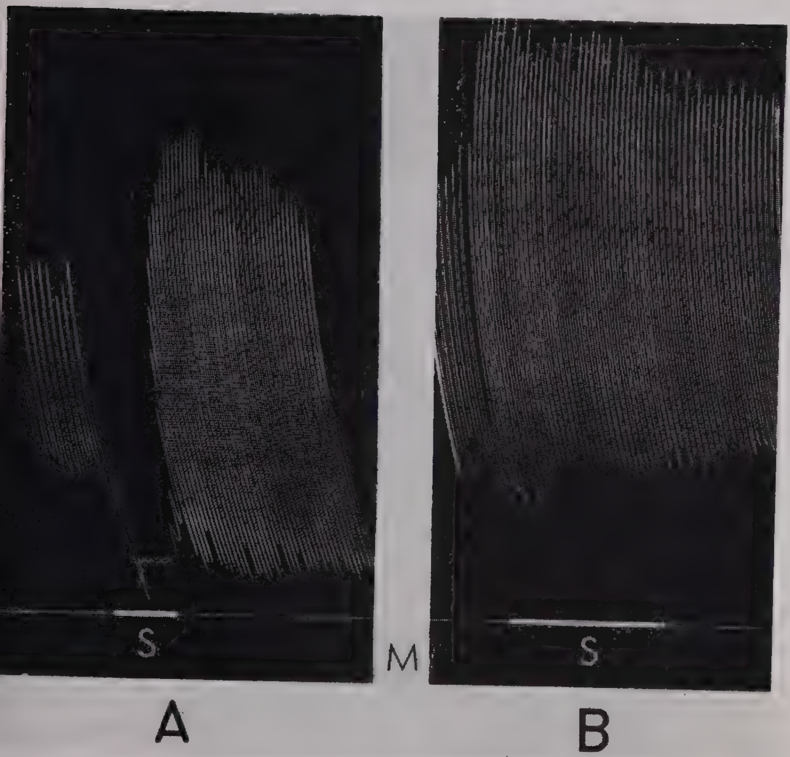

FIGURE 1. Effect of electrical stimulation of the visceral ganglion of *Venus mercenaria* on the beat of the emi-intact heart. (A) Inhibition produced by a 30-second stimulation at *S*, using a Harvard inductorium. Note ne greatly augmented amplitude following the period of stimulation. (B) Between records *A* and *B*, a few rops of 10^{-5}M mytolon were applied to the heart. After 10 minutes, a second period of stimulation identical ith the first, except for duration, produced excitation only.

; a greatly increased frequency and amplitude. A good example of this is een in FIGURE 1A. This has been taken as suggestive evidence that the cardioegulator nerves to the heart of *V. mercenaria* are mixed nerves and that reased acetylcholine for a time has a dominant inhibitory effect (Welsh and locombe, 1952). On cessation of stimulation, inhibition is rather quickly eplaced by excitation, which could result from the greater stability of an extor neurohumor. If the acetylcholine action could be blocked, one might e only excitation upon stimulation of the visceral ganglion. Such is the case, ; seen in FIGURE 1B. Mytolon (benzoquinonium) is a highly effective antagnist of acetylcholine on the heart of *V. mercenaria* (Luduena and Brown,)52). After treatment of the heart with 10^{-5}M mytolon, stimulation of the sceral ganglion produces excitation only.

That 5-HT has a marked excitor action on the heart of *V. mercenaria* has

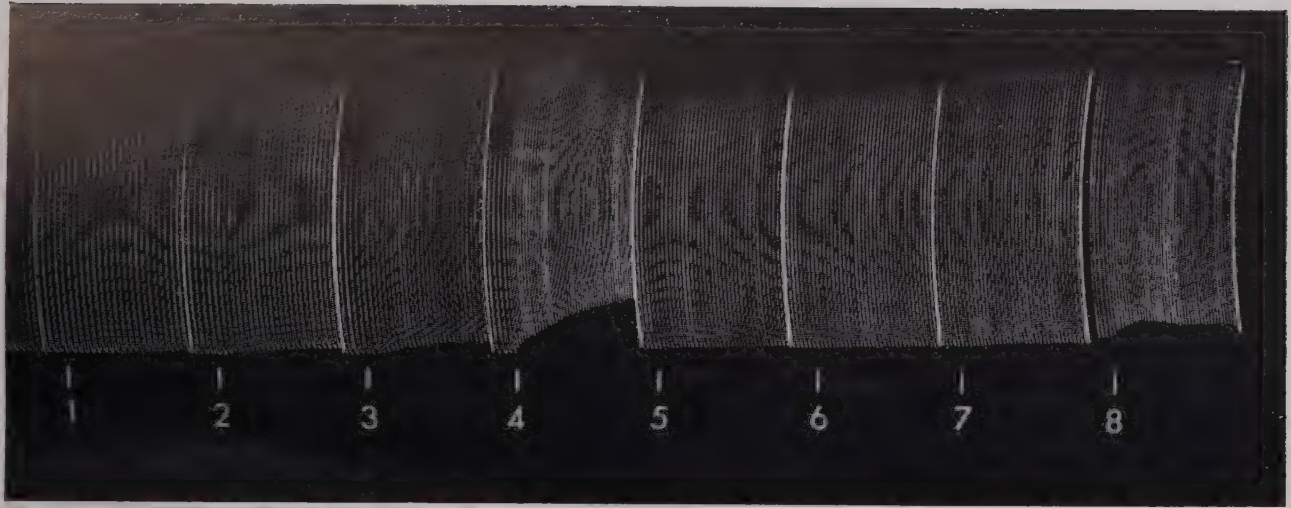

FIGURE 2. Effects on the isolated heart of *Venus mercenaria* of 5-HT alone and in the presence of dihydroergotamine (DHE). Volume of heart bath = 10 ml. (1) 10^{-8}M 5-HT; (2) 10^{-7}M 5-HT; (3) 10^{-6}M 5-HT; (4) 10^{-5}M 5-HT; (5) 10^{-8}M DHE followed by 10^{-8}M 5-HT; (6) 10^{-7}M DHE followed by 10^{-7}M 5-HT; (7) 10^{-6}M DHE followed by 10^{-6}M 5-HT; (8) 10^{-5}M DHE followed by 10^{-5}M 5-HT.

been shown earlier (Welsh, 1953a). In FIGURE 2 may be seen the actions of several concentrations of 5-HT alone, and then its actions following the addition to the bath of concentrations of dihydroergotamine (DHE) equimolar with a given dose of 5-HT. It is seen that 5-HT has a graded action over a wide concentration range, also that its action is partly blocked by DHE. It may be noted, however, that DHE increases the amplitude of the beat to such a degree that further excitation by 5-HT is greatly limited. The ability of the ergot alkaloids to excite the heart of *V. mercenaria* was first reported by Welsh and Taub (1948). It was confirmed by Tower and McEachern in the same year.

If acetylcholine has 5-HT as its opposing neurohumor in *V. mercenaria*, it should be possible to find these substances in the ganglia of this mollusk. In a series of experiments, pooled visceral, cerebropleural, and pedal ganglia of *V. mercenaria* were ground in eserinized sea water. The homogenate was heated at 100° C for 5 minutes and centrifuged, and diluted aliquots were tested on hearts of *V. mercenaria* that had been pretreated with lysergic acid diethylamide (LSD). LSD produces a maximal increase in amplitude without altering the sensitivity of the heart of *V. mercenaria* to acetylcholine. Such treatment permits the quantitative estimation of acetylcholine when excitor amines are also present in the extract.

One sees in FIGURE 3A a typical assay record when estimating acetylcholine. In a series of 6 experiments we obtained an average value for acetylcholine equivalent to 2 µg. AchCl per gm. wet weight of ganglion tissue.

Aliquots of ganglion homogenate were brought to pH 9 and heated at 100° C. for 5 minutes to destroy acetylcholine. These were assayed on hearts of *V. mercenaria* pretreated with mytolon as an extra precaution to prevent interference from acetylcholine. In FIGURE 3B is a typical assay record. A series of 6 separate experiments gave an average value of 15 µg. 5-HT per gm. wet weight of ganglion tissue.

In a comparable set of 6 separate determinations on pooled ganglia of the large gastropod, *Busycon* (*Fulgur*) *canaliculatum*, we found an equivalent of 5 µg. AchCl per gm. of tissue and of 17 µg. 5-HT per gm. of tissue. This value for 5-HT and the corresponding value given for *Venus* are perhaps too high. Erspamer and Boretti (1951) describe the activation of "enteramine I" by heating in an alkaline medium. It is possible that our method used to destroy acetylcholine converted an inactive precursor into active 5-HT.

As a further step in the identification of the excitor substance from ganglia of *Venus*, extracts were tested on the atropinized, estrous uterus of the rat in a manner suggested by Erspamer (1952) and Gaddum and Hameed (1954). Pooled ganglia of *Venus* were minced finely and acetone was added. After standing, the acetone was decanted and removed by reduced pressure. The dry residue was washed with petroleum ether, and the water-soluble materials were taken up in distilled water. The volume was adjusted so that material rom 100 mg. of wet tissue was taken up in 1 ml. distilled water. The action of such extracts was compared with known 5-HT on atropinized, estrous rat uteri. A typical record is seen in FIGURE 4. Here the material from the ganglia represented 20 mg. of tissue in each ml. of bathing fluid. Its activity was

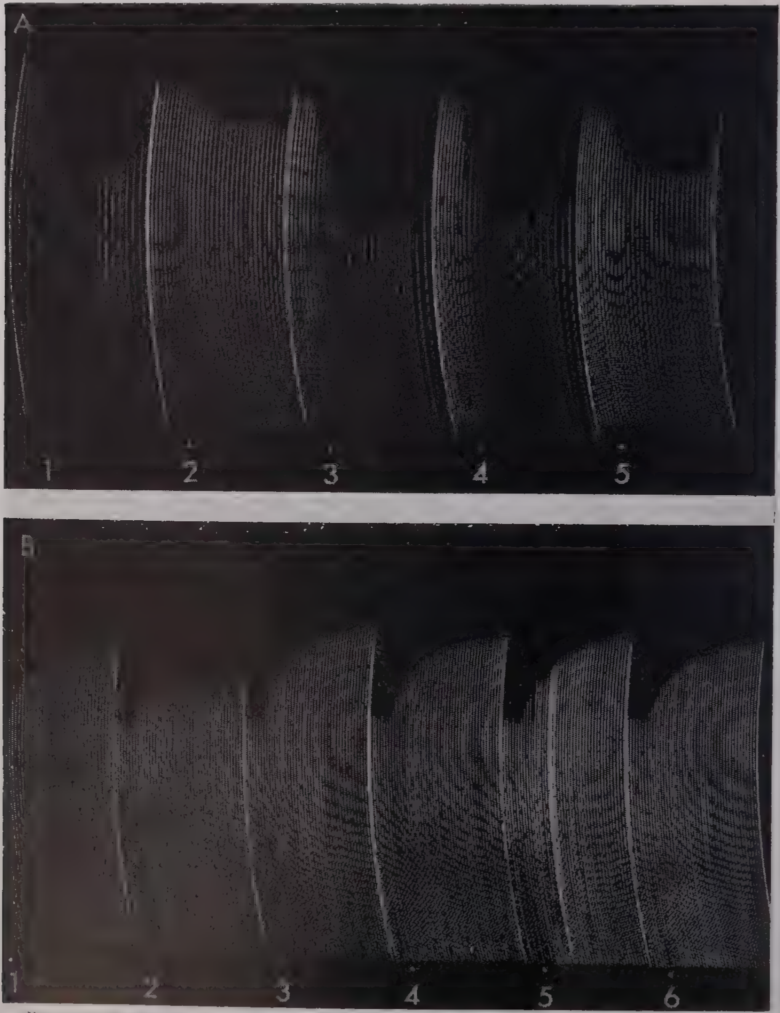

FIGURE 3. Records of actions of homogenates of pooled ganglia of *Venus* compared with acetylcholine (Ach) and 5-HT on the heart of *Venus mercenaria.*

(A) Ganglia homogenized in sea water plus eserine (1:10^5); heated at 100° C. for 5 minutes, diluted to give 4 mg. tissue/ml. Heart pretreated with 10^{-6}M LSD. (1) 2-ml. homogenate added to 10-ml. bath; (2) 10^{-9}M AchCl; (3) 5×10^{-9}M AchCl; (4) 2-ml. homogenate; (5) 4×10^{-9}M AchCl.

(B) Second heart of *Venus mercenaria* with 10^{-7}M mytolon present in bath at each test. Same homogenate as in *A*, but brought to *p*H 9 with NaOH and heated for 5 minutes. (1) 2-ml. homogenate added to 10-ml. bath; (2) 10^{-8}M 5-HT; (3) 10^{-7}M 5-HT; (4) 7×10^{-8}M 5-HT; (5) 2-ml. homogenate; (6) 8×10^{-8}M 5-HT.

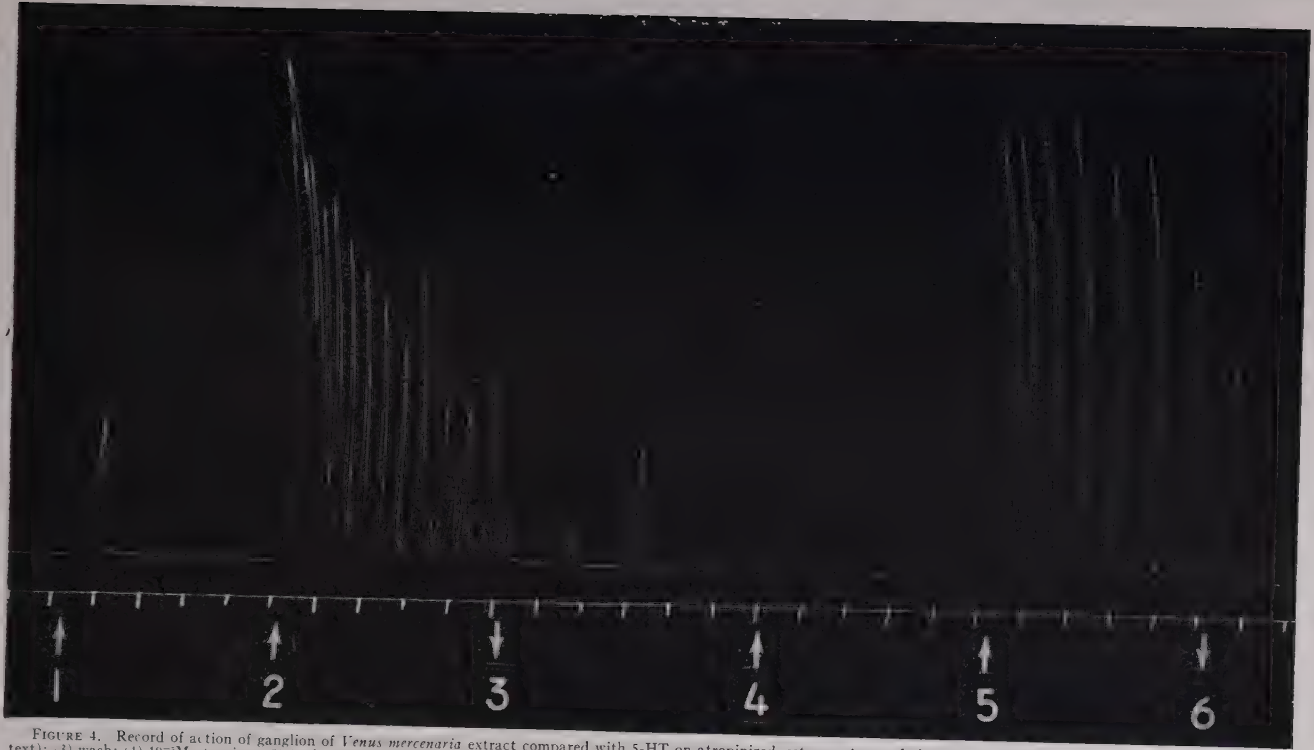

FIGURE 4. Record of action of ganglion of *Venus mercenaria* extract compared with 5-HT on atropinized, estrous uterus of the rat. (1) 10^{-5}M atropine; (2) ganglion extract (see text); (3) wash; (4) 10^{-5}M atropine; (5) 1 ml 10^{-5}M 5-HT in 20-ml. bath.

slightly greater than 5 × 10^{-7}M 5-HT. This gives a value of 4.4 μg. 5-HT per gm. of fresh tissue. Other determinations with the rat uterus were of this order. Such values might be expected in view of the different method of extraction.

In order to obtain more conclusive evidence concerning the identity of the 5-HT-like material, we resorted to chromatography. Ganglion tissue was extracted with acetone, the acetone was removed, and the dry residue was washed with petroleum ether. To the remaining material a small volume of 80 to 90 per cent ethanol was added. This alcohol extract was spotted on paper. The earlier chromatograms were run in butanol (100 parts), glacial acetic (30 parts), distilled water (90 parts). They were sprayed with a solution of the diazonium salt of *p*-nitroaniline according to Erspamer and Boretti (1951) or with bichromate-formaldehyde according to Shepherd, West, and Erspamer (1953). A sample set of chromatograms of extracts of the ganglia of *Venus* and *Busycon* is reproduced in FIGURE 5. Here the Rf value of the extract spot, assumed to be 5-HT, is seen to be slightly lower than that of known 5-HT. In the set from *Busycon*, however, it is seen that the added 5-HT ran with the material assumed to be 5-HT.

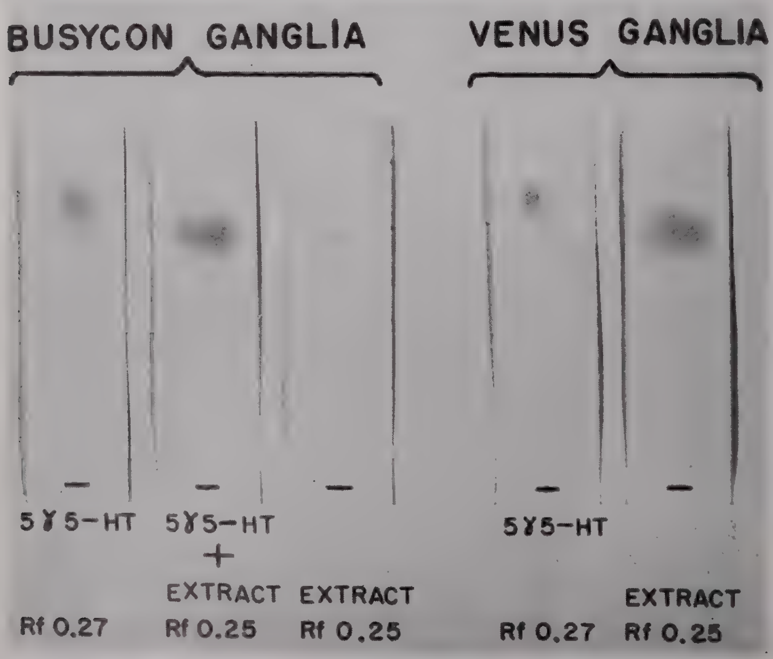

FIGURE 5. Portions of chromatograms of extracts of ganglia of *Busycon* and *Venus*. Solvent = butanol-acetic acid-H_2O.

We have recently chromatographed extracts of nerve tissue from other species of molluscs, using other solvents and other methods of visualizing the spots. It appears from the results that 5-HT may be found in the nervous systems of all classes of molluscs. The identification should be made more certain, however, through the use of newer methods of identifying 5-HT that make use of its strong fluorescence in HCl when activated by ultraviolet light (Udenfriend *et al.*, 1955; Bogdanski *et al.*, 1956). Using methods of bioassay and a chemical test, Florey and Florey (1953, 1954) had found that the ganglia of *Sepia*, a cuttlefish, contained considerable quantities of a substance having properties like 5-HT.

Evidence from the Byssus Muscle of Mytilus

Twarog (1954) has shown that 5-HT causes prompt relaxation of tonic contractions of the anterior retractor byssus muscle (ARBM) of *Mytilus*. Sustained contractions produced by acetylcholine, cathodal pulses, or mechanical stimulation are equally well relaxed by 5-HT. This muscle (or possibly nerves ending within it) was found by B. M. Twarog to contain a substance with the biological properties of 5-HT.

Recently Twarog (personal communication) has recorded simultaneously the demarcation potential of the ARBM and its mechanical response. For this she has used a 2-channel Grass ink writer with a D.C. amplifier for the electrical recording, and a strain gauge for the tension recording. She has kindly furnished a record for this paper and it is reproduced in FIGURE 6. One sees that depolarization (upper trace) produced by 10^{-5}M acetylcholine slightly precedes the increase in tension (lower trace). When the acetylcholine was washed off the muscle, there was fairly rapid repolarization, but only a slight drop in tension. Application of 10^{-7}M 5-HT perhaps hastened repolarization and resulted in a rapid decline in tension to a value somewhat below the starting value.

Twarog (1954) suggested that 5-HT plays a role in the inhibitory system of the ARBM of *Mytilus*. There is no new evidence that would contradict that view.

LSD and the Heart of Venus

A number of indole derivatives have been tested on the heart of *Venus*. Many act like 5-HT, and certain closely related compounds (N-methyl and N-dimethyl derivatives of 5-HT) have been found to be fivefold to tenfold more ctive than 5-HT (Bumpus and Page, 1955). 1-Tryptophan and indole acetic cid have an inhibitory action on the heart of *Venus*, which is quite the opposite of that produced by 5-HT (Welsh, 1953a).

Of the compounds that mimic the action of 5-HT on the heart of *Venus*, he most remarkable is lysergic acid diethylamide (LSD). On certain isolated molluscan hearts such as those of *Cyprina* and *Buccinum*, LSD was found to e a suitable antagonist to 5-HT and did not, by itself, greatly increase the mplitude and frequency of heartbeat (Welsh, 1954, 1956). On the heart of *Venus*, however, LSD, even in very low concentrations produces in time a

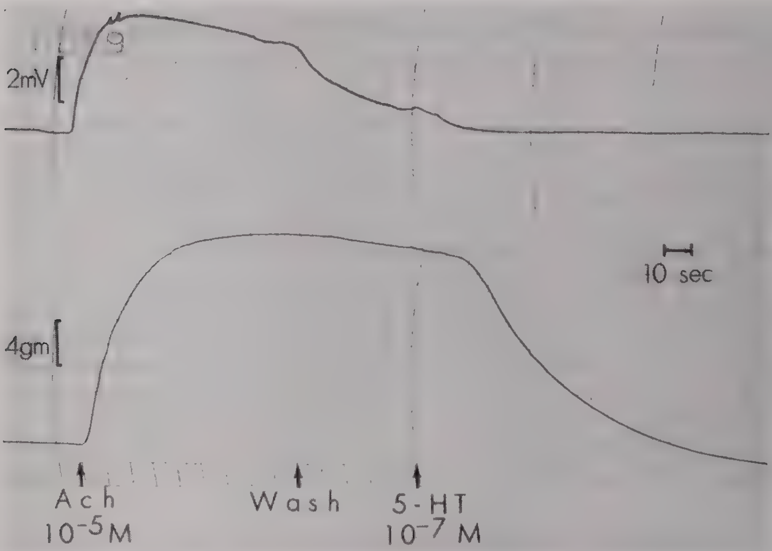

FIGURE 6. Simultaneous records of tension and demarcation potential in the anterior byssus retractor muscle. Depolarization follows washing, but the tension is sustained until the application of 5-hydroxytryptamine induces relaxation (record by B. M. Twarog).

maximal increase in amplitude and frequency of heartbeat. After washing a treated heart for many hours, the beat fails to return to normal.

FIGURE 7 is a record comparing the effects of equimolar amounts of 5-HT and LSD on the heart of *Venus*. It will be noted that the time required for each compound to produce its maximal effect is about 10 minutes at 10^{-7}M concentration (bath = 10 ml. capacity). This heart returned to normal, however, in about 10 minutes after washing out the 5-HT, but during 30 minutes of an attempted washing out of the LSD, the heart, if anything, showed a further increase in amplitude.

While this effect of LSD is no proof that the heart of *Venus* is normally regulated by 5-HT, it is an observation of interest in connection with the publication of this monograph. A more detailed study of LSD and the heart of *Venus* has been made, and the results will be published elsewhere.

Evidence from the Crustacea

Florey and Florey (1953, 1954) found in cerebral ganglia, ventral ganglia, and leg nerves of a variety of crustaceans a substance with such properties that they concluded it was 5-HT. It acted on the rat uterus, guinea pig ileum, crustacean heart, and closer muscle of the crustacean claw in the manner of 5-HT. Extracts when treated with diazonium salt of *p*-nitroaniline gave a color like that obtained with known 5-HT.

When extracts of different parts of the nervous system of *Dromia*, a crab,

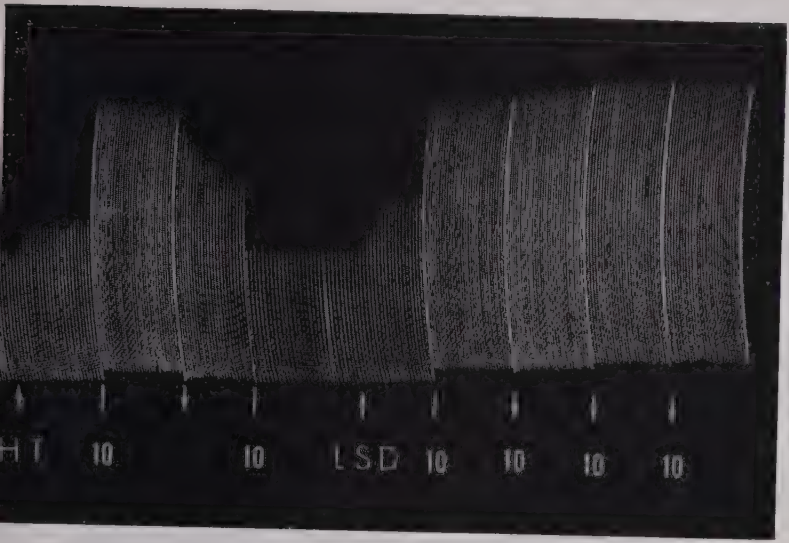

Figure 7. Record comparing the actions of equimolar concentrations of 5-HT and LSD on the isolated heart
of *Venus mercenaria*. HT = 10^{-7}M 5-HT; LSD = 10^{-7}M LSD. At the vertical bars the drum was stopped for
0 minutes. At the downward-pointing arrows, drum was stopped for 10 minutes and the heart was washed re-
eatedly. Volume of bath = 10 ml.

vere assayed on the atropinized rat uterus, the Floreys obtained effects such
s those produced by equivalents of 18.2–22.5 μg. 5-HT/gm. dry weight of
issue.

There exist in the pericardial cavity of decapod crustaceans complex net-
vorks of fine nerve endings. They are disposed in such a manner that, in
ome species, blood entering the cavity from the gills is made to flow past
hese endings before it enters the heart via the ostia. Alexandrowicz (1953)
rst described these networks in detail and proposed that they be called peri-
ardial organs. He set forth evidence that these organs produce a substance
hat influences the heart rhythm. Alexandrowicz and Carlisle (1953) extracted
ericardial organs and tested the extracts on hearts of 4 species of crustaceans.
n 3 species (*Cancer pagurus*, *Homarus vulgaris*, and *Squilla mantis*) the action
f the extract resembled that produced by adrenaline or noradrenaline. In
Iaia squinado, however, the extracts decreased the frequency of beat, while
drenaline and noradrenaline accelerated the beat as in the other species.

A limited number of experiments were done at the Plymouth Laboratory,
lymouth, England, in the summer of 1953 by the author in collaboration with
lexandrowicz and Carlisle. Actions of extracts of pericardial organs and
ıown tryptamine and 5-HT were compared on a molluscan heart and on a
ustacean heart. The effects of extracts and the indole amines were similar,
HT being more active than tryptamine. Taken by themselves, however,
ese experiments do not identify with certainty the material of the pericardial
gans that appears to be a cardioregulator substance in crustaceans.

Florey and Florey (1953, 1954) also found that 5-HT had a marked excito action on the hearts of the crustaceans with which they worked. They obtained a threshold for 3 species between 10^{-8} and 10^{-9} gm. of 5-HT per ml. o perfusing solution. Adrenalin had a similar action, but only at 100 time the concentration of 5-HT.

With the collaboration of D. M. Maynard, Jr., the action of 5-HT has bee tested on isolated hearts of several New England species of decapod crustaceans. Some species are more sensitive than others. One with a low threshold is *Cancer borealis*, the Northern or Jonah crab. The heart of this crab responds by an increase in frequency to concentrations of 5-HT between 10

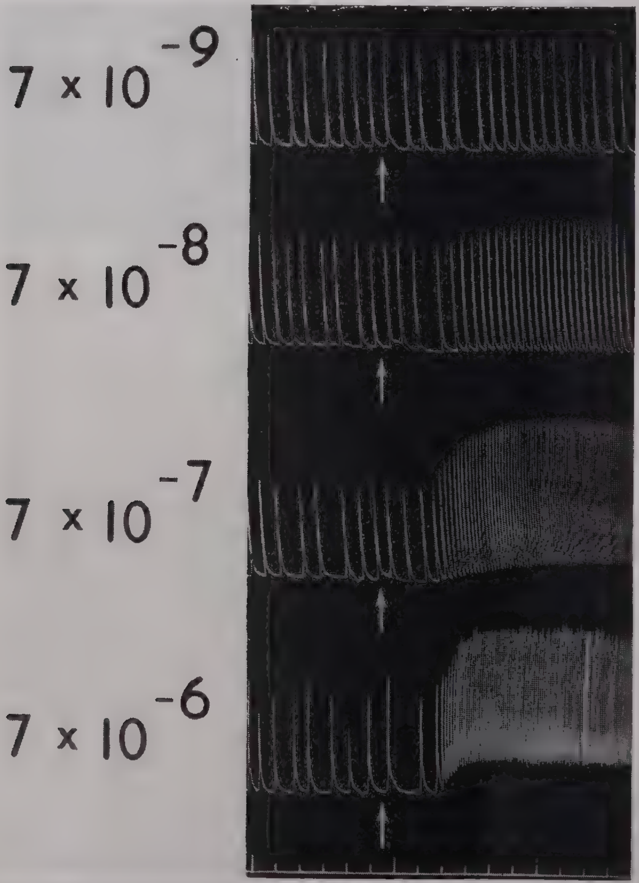

FIGURE 8. Record of the action of 4 concentrations of 5-HT on the isolated heart of *Cancer borealis*, a crab. Arrows indicate point at which 0.5 ml. of 5-HT was injected into the perfusion line leading to the heart. Concentrations indicated at left are of injected solutions. Actual concentration in heart unknown, but lower. Time interval = 10 sec. (record by D. M. Maynard, Jr.).

and 10^{-9} molar. FIGURE 8 gives recordings of the effects of 4 different concentrations of 5-HT. At the highest concentration a remarkably sudden increase in frequency is seen, especially since the arrow indicates the point at which the 5-HT was injected into the perfusing system at some distance from the heart. The 5-HT probably acts on the cardiac ganglion to reduce the interval between "bursts" of discharges from the neurons that act as pacemakers for the heart. For a discussion of the electrical activity of the crustacean cardiac ganglion the reader is referred to a recent paper by Maynard (1955).

On the basis of our present evidence it appears that 5-HT or a closely related indole amine is a cardio-regulator in decapod crustaceans. This is of particular interest since the heart of decapod crustaceans is neurogenic and provides readily accessible neurons. A study of the effects of applied 5-HT and various related compounds on these neurons should be of considerable value in gaining a better understanding of the mechanism of action of 5-HT at the cellular level.

Comment

Much remains to be done before a neurohumoral role for 5-HT in the lower animals is clearly established. Among the vertebrates it would appear that only in the mammals has the occurrence of 5-HT in the nervous system been demonstrated. The effects of 5-HT have been observed on a few of the lower vertebrates. Erspamer (1954a) reports a weak and variable constrictor action on the blood vessels of the hind legs of a frog and of a toad. He states (1954b) that, at that time, no experiments had been published on the effects of 5-HT on the isolated frog's heart. Vogt (1954) finds that the isolated frog's rectum responds to low concentrations of 5-HT (threshold below 1 in 10^8) by an increase in tone and the appearance of rhythmic movements.

In the invertebrates little has been done with groups other than the mollusks and crustaceans to determine the occurrence of 5-HT in the nervous system or to ascertain its actions on isolated organs. Within the mollusks 5-HT is a highly effective excitor agent on the hearts of several species, while it relaxes such noncardiac muscle as that controlling the tension on the byssus threads of *Mytilus* (Twarog, 1954) and the radula muscles of *Busycon* (Robert Hill, unpublished). Muscle in the mollusks is of the smooth type. Although it may be physiologically specialized for differing tasks and may even show signs of striation in places where it is fast acting (for example, as a fast closer of scallops and in certain cephalopod hearts), it is basically like vertebrate smooth muscle in its innervation and its response. If a high sensitivity to a naturally occurring amine can be taken as significant, then certainly some molluscan muscle responds to far lower doses of 5-HT than of adrenalin or noradrenalin (Gaddum and Paasonen, 1955).

Perhaps the most important step to take next in a study of the role of 5-HT as a neurohumoral agent in the invertebrates is to apply methods that more certainly identify it. Such methods of identification as those recently developed at the National Heart Institute by Udenfriend and others should be ulitized.

Summary

5-Hydroxytryptamine occurs in a variety of non-nervous tissues in the lower vertebrates and in the invertebrates. In some instances it is a constituent of venoms. This indole amine also appears to be present in the nervous systems of mollusks and crustaceans. There is evidence that 5-HT is a product of cardioregulator nerves in mollusks and crustaceans where it is an excitor agent. In the former group, 5-HT relaxes certain noncardiac muscle and appears to be a product of some inhibitor nerves. The heart of *Venus mercenaria* shows a pattern of drug action that supports the view that it is normally regulated by 5-HT or by a closely related compound.

Acknowledgments

The author is especially obligated to Anne McCoy for technical assistance in connection with the work here reported. 5-Hydroxytryptamine creatinine sulfate was kindly supplied by the Abbott Laboratories, Chicago, Ill., mytolon by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., and ergot alkaloids by Sandoz Pharmaceuticals, Hanover, N. J.

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A CONCEPT FOR A ROLE OF SEROTONIN AND NOREPINEPHRINE AS CHEMICAL MEDIATORS IN THE BRAIN

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Public Health Service, Department of Health, Education,
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It is not the purpose of this paper to make a survey of the work suggesting that serotonin is a central neurohumoral agent, since much of this is being discussed by other contributors to this monograph. Rather, we should like to offer a concept that implicates serotinon and norepinephrine as chemical mediators of mutally antagonistic centers in the brain. This thesis, which is admittedly oversimplified, endeavors to explain the actions of the tranquilizing agents reserpine and chlorpromazine and the hallucinogenic agents LSD and mescaline in terms of interactions with serotonin and norepinephrine in the central nervous system. As presented here this concept may begin to trace the outlines of a coherent picture, but one, we are sure, that will bear only a faint resemblance to the picture that will ultimately emerge. The concept is only a working hypothesis, but it has already proved useful in linking a number of unrelated observations and in suggesting certain experiments that might test it.

It is fascinating to learn how the discovery of serotonin, a substance that appears to have no part in the general metabolism of cells, has proved to be of such significance to the pharmacologist, the biochemist, the neurophysiologist and, possibly, to the psychiatrist. When the vasoconstrictive material in blood platelets was finally isolated and identified as 5-hydroxytryptamine,[1, 2] it was soon proved identical with the enteramine that Erspamer[3] had extracted years before from the gastrointestinal tracts of the vertebrates and from other organs in the invertebrates. A number of notions concerning its role in the body were considered, most of which took into account its profound contractive action on smooth muscle. Among these possibilities were the control of vascular tone and, therefore, of systemic blood pressure; control of gastrointestinal motility; regulation of water excretion by affecting kidney arterioles; and regulation of hemostatic action by affecting blood vessels after release from platelets.[3, 4] Several of these suggestions can be more or less summarily dealt with at once. Since serotonin is normally present in the body almost entirely in a bound and therefore presumably inactive form, it is doubtful that there is enough of the free form circulating in plasma to affect the vascular tone either of the body as a whole or of the kidneys in particular. Results from our laboratory indicate that it is not involved in any obvious way in hemostasis, since animals and humans whose platelets have been depleted of serotonin by the administration of reserpine show no change in bleeding or clotting time.[5] It is possible that serotonin controls some aspects of gastrointestinal motility, although no direct evidence is as yet forthcoming in this connection.

The clue pointing to a role for serotonin in the functioning of the central nervous system came from its discovery in the brain.[6, 7] The observation by Gaddum[8] that extremely low concentrations of lysergic acid diethylamide (LSD) counteracted the constrictive action of serotonin on certain smooth-muscle preparations prompted him to propose that LSD might also antagonize an essential action of serotonin normally present in the brain and thus provoke the mental disturbances observed in man. In this country Woolley and Shaw[9] presented the idea that mental diseases might result from an imbalance in the amount of serotonin in the brain.

The paper by Shore *et al.* in this monograph presents experimental evidence indicating that the primary action of reserpine is to impair serotonin binding sites so that they lose, in part, their capacity to retain serotonin. As a result, serotonin made in the brain, instead of being stored, remains in a free, pharmacologically active form and presents to brain tissue a persistent low concentration of free serotonin. It is this free serotonin that is considered to exert the actions attributable to reserpine.

The rapid disappearance of reserpine from the brain in contrast to its persistent pharmacological effects and the persistent change in brain serotonin have provided a valuable tool in ascertaining what central actions of reserpine are mediated through serotonin. Following the administration of 1 to 5 mg./kg. of reserpine to rabbits, dogs, or mice, the following effects were observed: sedation, generalized reduction in motor activity, decrease in depth and rate of respiration, potentiation of hypnotics, and a series of parasympatheticlike effects, including a reduction in the arterial blood pressure, slowing of the heart rate, lowering of the body temperature, constriction of the pupils, ptosis, relaxation of the nictitating membrane, increased lacrimation, and salivation. In addition there was an increased motility of the gastrointestinal tract. It is possible that the latter effect is mediated peripherally through the persistent free serotonin in the intestines. These effects were observed for a period of almost 48 hours following the injection of reserpine. Thus the action of the drug appears to bear a temporal relationship to the change in brain serotonin rather than to the concentration of reserpine. These data, together with other observations reported by Shore *et al.*, provide strong evidence that the effects of reserpine are caused by the actions of free serotonin. From these results it may be inferred that serotonin is normally involved in the regulation of centers in the brain that involve wakefulness, temperature regulation, blood-pressure regulation, and a number of other autonomic functions.

The question arises as to how serotonin in the brain is normally involved in these functions. The diversity of actions associated with serotonin makes it tempting to consider the possibility that it is a synaptic transmitting agent acting on the brain centers in the hypothalamus and other subcortical centers.

Few problems are more difficult to investigate in clear-cut fashion than the possibility that a chemical substance is a transmitter of impulses across synaptic junctions in the brain. The brilliant researches of Otto Loewi have made the problem relatively simple in the peripheral nervous system, where ganglia can be isolated and perfused and where, after stimulation of preganglionic fibers, the perfusate can be examined for a neurohumoral agent. Thus it is now generally accepted that synaptic transmission in autonomic ganglia and transmission at the neuromuscular junction are mediated by acetylcholine. Direct proof is still lacking for a similar role for acetylcholine centrally, however, although for many years the substance has been known to be present in the central nervous system.

There are provocative suggestions that serotonin is a chemical mediator in the brain. Like acetylcholine, the substance is present in nervous tissue in a precursor state and, as indicated by Shore *et al.*, it seems to be active only in a free form. The amine is unevenly distributed in brain, its concentration being highest in the brainstem, especially the hypothalamus, lowest in the cortex, and almost undetectable in the cerebellum, as indicated by Amin *et al.*[7] and by Udenfriend *et al.* in this monograph. A high biological activity with a predilection for the brainstem is strong indication that a substance has physiological significance in the specialized functions of this region. Thus we find that reserpine acts on the brainstem, apparently through free serotonin. This makes it plausible that the amine acts as a mediator for certain subcortical centers. Collateral support for a role of serotonin in nerve transmission is provided by the distribution of monoamine oxidase, the enzyme that destroys it, and 5-hydroxytryptophan decarboxylase, the enzyme that synthesizes it, both of which have been reported by Gaddum and Giarman[10] and by Udenfriend *et al.* elsewhere in these pages, to be in highest concentration in the hypothalamic areas. A strong argument for a neurohumoral role for serotonin in certain invertebrates has been brought forth in this monograph by Welsh's presentation of his work with the mollusks.

Chemical mediators such as acetylcholine are considered to work through the membrane depolarization of nerve cells. Thus a nerve impulse along a peripheral preganglionic fiber results in the liberation at the ganglion cell of free acetylcholine from a precursor complex. This agent diffuses across the synapse and, by depolarizing the postganglionic nerve-cell body, is instrumental in the transfer of the impulse to the other side of the junction. If a cholinesterase inhibitor is present, the acetylcholine released is protected from destruction and may produce a volley of postganglionic impulses. If an excess of cholinesterase inhibitor or acetylcholine is present, however, the ganglion cell is flooded with acetylcholine, which results in persistent depolarization and thus prevents activation of the postganglionic nerve by preganglionic stimulation.[11] Accordingly, an excess of acetylcholine can counteract its own action.

Similarly, in the brainstem, nerve impulses along presynaptic fibers might release serotonin in minute amounts at synaptic junctions. This release of free serotonin might act as a chemical mediator of responsive brain centers that in turn send impulses along postsynaptic fibers. Ordinarily, free serotonin could be considered to be present at the synapse for only a short time following presynaptic impulses. Reserpine, by impairing the sites that hold serotonin in a bound form without blocking the synthesis of the amine, would present a low but persistent concentration of free serotonin in direct contact with the brain center. As a consequence, a continuous volley of impulses would bombard the center, resulting in the usual manifestations of reserpine action.

What would happen if "excess" free serotonin were to flood the brain centers

TABLE 1

EFFECT OF IPRONIAZID ON THE CONCENTRATION OF SEROTONIN IN BRAINS OF NORMAL AND RESERPINE-TREATED RABBITS*

Drug	Brain serotonin	Effect
	μg./gm. (average)	
None	0.55	—
Reserpine	0.10	Sedation
Iproniazid	0.63	None
Iproniazid followed by reserpine	0.42	Excitement
Reserpine followed by iproniazid	0.10	Sedation

* The rabbits were given 5 mg./kg. of reserpine intravenously. Iproniazid (100 mg./kg.) was given intravenously 2 hours before or after reserpine. The animals were sacrificed 1 hour after administration of last drug.

excited by the amine? Like acetylcholine, would it counteract its own action? A large amount of free serotonin in the brain was induced in 2 ways. Rabbits were pretreated with 100 mg./kg. of iproniazid (Marsilid), a compound that inhibits monoamine oxidase and consequently the metabolism of free serotonin. Two hours later the animals were given, intravenously, 5 mg./kg. of reserpine to release the brain serotonin. Control animals given iproniazid alone evinced no overt symptoms and, within the time limits of the experiments, little change in brain serotonin. Animals pretreated with iproniazid and then given reserpine showed only a small decline in serotonin, which indicated that the released amine had not been metabolized (TABLE 1). The pharmacological effects were dramatic. Instead of having the usual reserpine effects, the rabbits were now considerably excited (FIGURE 1). In addition, as is shown in TABLE 2, they showed all the sympatheticlike effects produced by LSD.[12]

Thus excess free serotonin, like excess free acetylcholine, seems to counteract its own action. Apparently, however, it does more than this, since the resulting signs are identical with those produced by the hallucinogen LSD. These signs are the opposite of those observed after reserpine. It was these results that first suggested to us that the blocking of serotonin action at certain brain centers unmasked a mutually antagonistic system of brain centers.

Udenfriend *et al.*, in this same monograph, found another way of increasing the concentration of free serotonin in the brain. If rabbits or dogs are given 5-hydroxytryptophan, this compound passes rapidly into the brain, where it is decarboxylated over a considerable period of time to form serotonin. Small doses showed no action or appeared to have a sedative effect. If the dose of 5-hydroxytryptophan was large enough, serotonin was formed in high concentration and, presumably by saturating its binding sites, resulted in a high concentration of free amine. Again the animals were excited and behaved almost exactly as though they had received LSD (TABLE 2).

It is of interest that similar central effects can be induced both by a functional deficiency of free serotonin, presumably brought about by LSD, and by an excess of free serotonin. LSD may be considered to block nerve conduction by interfering with the normal depolarizing action of serotonin released at synaptic junctions. Excess serotonin may be considered to block conduction by inducing a persistent state of depolarization. In both situations presynaptic impulses would be ineffective and would not evoke postsynaptic responses.

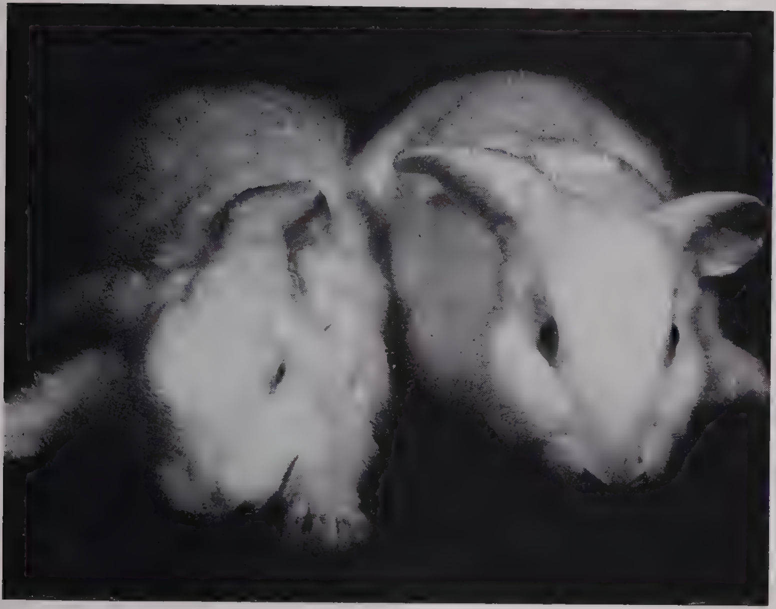

FIGURE 1. Effect of iproniazid plus reserpine on 2 rabbits. Both animals were given reserpine (5 mg./kg. intravenously) but the animal on the right was pretreated with iproniazid (100 mg./kg.).

A problem that has been of some concern to us is how reserpine, which acts through serotonin, and chlorpromazine, which does not act through serotonin, can produce similar central effects, including sedation and potentiation of hypnotics. A number of observations make it evident that reserpine and chlorpromazine induce similar effects in the brain, but do so by different mechanisms. On the one hand, the action of reserpine has been shown to be irreversible in that its effects last far beyond the time the drug is detectable in tissues, whereas chlorpromazine does not affect the serotonin in cells and seems to act reversibly, that is, no effects are seen when the drug has disappeared from the body. A second difference may be seen in the nature of the potentiating action of the 2 agents on certain hypnotics. Both compounds have marked activity, but only that of reserpine is blocked by LSD (TABLE 3).

Still a third difference between the actions of the 2 substances has been shown. As described before, an LSD-like effect was induced by an excess of free serotonin in the brains of rabbits, whether produced by the administration of iproniazid followed by reserpine or by giving 5-hydroxytryptophan. If rabbits so treated were then given reserpine they did not become sedated (FIGURE 2a). It would not be expected that reserpine, if it acts through serotonin, would be a sedative agent in this situation. There is already too much free serotonin present. On the other hand, the administration of chlorpromazine rapidly sedated the animals (FIGURE 2b).

TABLE 2

COMPARISON OF THE PHARMACOLOGICAL EFFECTS OF RESERPINE (PERSISTENT "LOW" FREE SEROTONIN), LSD, "EXCESS" FREE SEROTONIN, AND AMPHETAMINE

Effect on:	Persistent "low" free serotonin (reserpine)	LSD	Persistent "excess" free serotonin (iproniazid + reserpine or 5-hydroxytryptophan)	Amphetamine
Alertness	Decrease	Increase	Increase	Increase
Motor activity	Decrease	Increase	Increase	Increase
Temperature	Decrease	Increase	Increase	Increase
Depth and rate of respiration	Decrease	Increase	Increase	Increase
Blood pressure	Decrease	Increase	Increase	Increase
Pupil	Constriction	Dilatation	Dilatation	Dilatation
Eyelid	Ptosis	—	—	—
Nictitating membrane	Relaxation	—	—	—
Heart rate	Decrease	Increase	Increase	Increase
Erection of hair	—	Piloerection	Piloerection	Piloerection
Visual	—	Apparently blind	Apparently blind	?

TABLE 3

EFFECT OF LSD ON ETHANOL POTENTIATION BY RESERPINE AND CHLORPROMAZINE*

Drugs	Duration of hypnosis†
	minutes (average)
Ethanol (controls)	40
Ethanol + reserpine	>300
Ethanol + LSD + reserpine	54
Ethanol + chlorpromazine	150
Ethanol + chlorpromazine + LSD	147

* The adult male mice were given the various drugs intraperitoneally. Reserpine (5 mg./kg.) was administered 1 hour before ethanol (4 gm./kg.). Chlorpromazine (5 mg./kg.) was given simultaneously with ethanol. LSD (10 mg./kg.) was given in 2 divided doses 1 hour before and simultaneously with ethanol. The mice given ethanol alone served as controls. LSD by itself had virtually no effect on the duration of the hypnosis produced by ethanol.

† Time from loss to return of righting reflex.

The indications that chlorpromazine and reserpine act centrally by a different mechanism are summarized in TABLE 4.

The difference in the mode of action of reserpine and chlorpromazine may be explained by assuming that they act on physiologically antagonistic systems in the brainstem that are involved in wakefulness, regulation of temperature, control of blood pressure, and other autonomic functions. Drug-induced paralysis of one system would release the opposite system and allow it to predominate (FIGURE 3). In support of this concept, it has been demonstrated that there are both parasympathetic and sympathetic areas in the brainstem. In the hypothalamus, one of the principal foci of integration of the entire autonomic system, one finds primarily parasympathetic representation in the forepart and sympathetic in the posterior part. In view of the effects of reserpine it may be supposed that serotonin is the chemical transmitter of nerve impulses

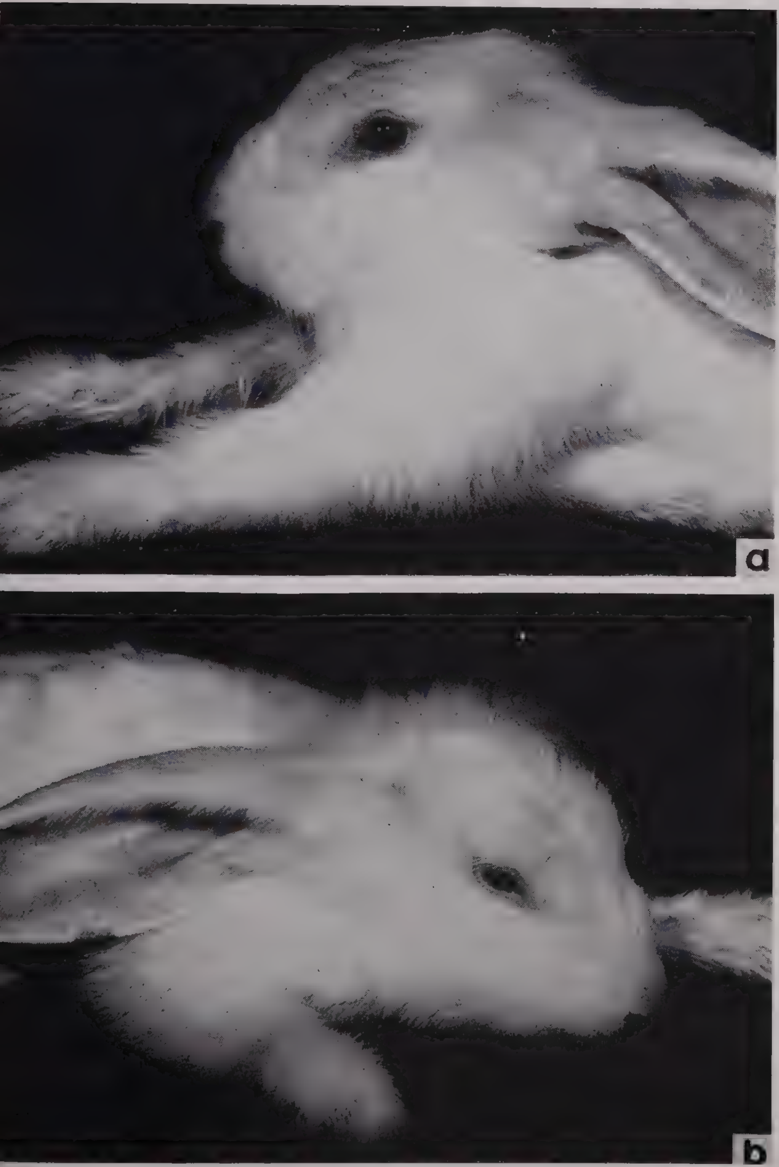

FIGURE 2. FIGURE 2a shows the effects of reserpine on a rabbit with excess serotonin in the brain. These nimals were pretreated with iproniazid (100 mg./kg.) followed 1 hour later by reserpine (5 mg./kg. intraveously). Thirty minutes later the excited animal was given a further administration of reserpine (5 mg./kg.). IGURE 2b shows the effects of chlorpromazine on a rabbit with excess serotonin in the brain. The animal was retreated with iproniazid (100 mg./kg.) followed in 1 hour by reserpine (5 mg./kg. intravenously). Thirty minutes later the excited animal was given chlorpromazine (5 mg./kg.).

Table 4

Summary of the Indications that Chlorpromazine and Reserpine Act Centrally by Different Mechanisms

	Reserpine	Chlorpromazine
Potentiation of hypnotics	Antagonized by LSD	Not antagonized by LSD
Effect on serotonin	Releases	Does not release
Duration of effect	Not related to brain level of drug	Related to brain level of drug
Effect on animal pretreated with iproniazid	Excitement	Sedation

to the centers of the parasympathetic division.* If this speculation should be correct, then perhaps fibers that innervate these centers should be termed "serotonergic nerves." By blocking serotonergic fibers, LSD would unmask the action of the opposing sympathetic system* and produce its typical sympatheticlike responses. Reserpine could be considered to invoke its parasympatheticlike effects by presenting a low but persistent concentration of free serotonin to activate the parasympathetic centers.

Chlorpromazine could be postulated to block nervous impulses activating central sympathetic centers and thus augment the activity of the parasympathetic system. This would account for the similarity of the central effects of chlorpromazine and reserpine, even though they act by different mechanisms. An analogy can be drawn from the peripheral autonomic system, where both norepinephrine and atropine cause dilatation of the eye, but by different mech-

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* The reciprocal systems are designated as sympathetic and parasympathetic, although it is recognized that these terms may be inadequate, since other centers involving wakefulness and appetite, though autonomic in the true sense of the word, do not involve the peripheral vegetative system directly.

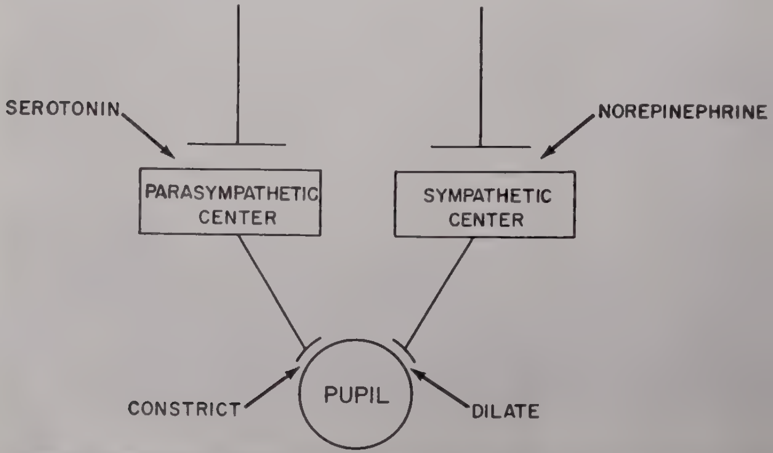

Figure 3. Schematic diagram depicting mutually antagonistic brain centers in the central autonomic nervous system.

anisms—one by direct stimulation of the effector organ and the other by blocking the action of the opposing system (antiacetylcholine) on the effector organ.

Chlorpromazine could be assumed to inhibit the sympathetic system by blockade of the chemical mediator that activates the sympathetic centers. What could this chemical mediator be? Norepinephrine is a suitable candidate, although the evidence for it is entirely circumstantial. First and most mportant is the fact that catecholamines, about 85 per cent of which are norepinephrine and the remainder epinephrine, are present in the brain. Vogt[13] has shown that norepinephrine is distributed in a manner similar to serotonin, being in highest concentration in the brainstem, especially the hypothalamus, owest in the cerebral cortex, and virtually absent in the cerebellum. From its uneven distribution and its localization in the parts of the brainstem that contain the sympathetic centers, Vogt has suggested that norepinephrine in the brain might have a transmitter role.

The concept of sympathetic centers, controlled by nerve fibers that release norepinephrine following nerve impulses, allows an interpretation of a number of observations previously difficult to explain. The first of these are the cenral actions that are observed after the administration of epinephrine or norpinephrine. These agents, administered parenterally in small doses, have elatively minor central actions, as little of the compounds would be expected o pass the blood-brain barrier. Large doses induce marked excitement in nimals, however, which could be explained by the activation of the sympahetic centers by the small amounts of the amines that enter the brain. The xcitement induced by these catecholamines would be the antithesis of the selation resulting from parenterally administered serotonin.

Some of the actions of ephedrine and amphetamine, hitherto not undertood, may be explained by assuming that these compounds have a direct acion on the various sympathetic centers in the brain. The peripheral actions f these compounds simulate those of norepinephrine with close fidelity, so hat they have often been considered to act directly on autonomic effector rgans. There is substantial evidence, however, which is not easily reconciled with the thesis of a direct action solely on the effector organs. First, the effects of ephedrine are generally less on isolated tissues than in the whole animal. econd, ephedrine and amphetamine do not evoke enhanced responses, as do he catecholamines, after sympathetic denervation of the effector organ. In act, the activity of ephedrine is markedly lessened by denervation. The exlanation has been offered that ephedrine and amphetamine do not act directly, ut indirectly by protecting the adrenergic mediator at sympathetic nerve erminals from the action of monoamine oxidase. This explanation is unsatisactory, since ephedrine and amphetamine exert only weak inhibitory effects n monoamine oxidase, whereas iproniazid, a much stronger inhibitor, does not voke obvious sympathomimetic effects.

A more satisfactory way of explaining the peripheral actions of ephedrine nd amphetamine is to assume that these substances mimic the action of norpinephrine centrally. Both drugs rapidly pass into the brain in high concenration,[14, 15] and it is conceivable that by activating the central sympathetic ystem they would indirectly affect peripheral organs. This would be in accord with the observations that sympathetic denervation considerably lessens the peripheral effects of ephedrine and amphetamine and would explain why the compounds are relatively inactive on isolated tissues.

It is of considerable interest that ephedrine and amphetamine produce practically the same spectrum of effects invoked by LSD, although considerably larger doses of the former drugs are required (TABLE 2). Large doses lead to excitement, and in man even to mental disturbances, including hallucinations. Mescaline (trimethoxyphenylethylamine), which is structurally related to epinephrine rather than serotonin, might be considered as another centrally acting compound that easily crosses the blood-brain barrier and mimics the action of norepinephrine. Mescaline, like epinephrine, causes anxiety, sympathomimetic autonomic effects, generalized tremors, as well as vivid hallucinations. Thus mescaline, which produces mental effects like those produced by LSD, may act not by blocking "serotonergic" brain centers but by stimulating the reciprocal "adrenergic" ones.

It is possible to interpret at least part of the peripheral autonomic effects of chlorpromazine in terms of the model presented in FIGURE 3. If chlorpromazine blocks the action of norepinephrine centrally, thus unmasking the parasympathetic centers, peripheral manifestations such as miosis should result in part from this central action. Experimental evidence cited in the literature supports the thesis that some of the peripheral adrenergic blocking effects of chlorpromazine are mediated centrally. Dasgupta and Werner[16] have demonstrated that chlorpromazine lowers blood pressure when given in doses that are too small to alter the pressor effects of administered epinephrine. For example, the injection of small doses of chlorpromazine intracisternally into monkeys causes a fall in blood pressure and a blockade of the carotid pressor reflex, while the pressor response of systemic injections of epinephrine remain unaffected. The same workers have shown with decorticated cats that the rise in blood pressure that results from electrical stimulation of hypothalamic and medullary pressor areas is abolished by doses of chlorpromazine too low (50 to 100 μg./kg.) to exert a peripheral adrenergic blocking effect. Finally, Jourdan *et al.*[17] state that chlorpromazine is inactive in the spinal dog at a dose that is hypotensive in intact animals. From these reports it would seem likely therefore that chlorpromazine may influence the circulatory system by a central action.

Of particular interest are the observations of Moran and Butler,[18] of this laboratory, who have shown that chlorpromazine sulfoxide, a major metabolite of chlorpromazine, produces sedation, orthostatic hypotension, and partial blockade of the carotid sinus reflex at doses that do not block the rise in blood pressure induced by injected epinephrine.

The actions of a number of other adrenergic blocking agents can also be interpreted in terms of inhibition of norepinephrine action at sympathetic brain centers. For example, the dihydro-ergot alkaloids, which are potent adrenergic blocking agents, usually induce sedation.

Dibozane, 1,4-bis(1,4-benzodioxan-2-yl-methyl)piperazine, an adrenergic blocking agent, was recently tested in man for its hypotensive action and discarded because of the considerable degree of sedation it produced, in many instances at doses that did not lower blood pressure.[19] We have found, interestingly enough, that it also potentiates the effects of barbiturates.

On the other hand, the dibenamine-type compounds do not seem to act as central adrenergic blocking agents. They do not alter the direct effects of epinephrine and other adrenergic stimuli on the central nervous system, nor do they prevent epinephrine-induced hyperventilation or the increased motor activity induced by certain sympathomimetic amines.[20]

There has been considerable interest in the possibility that disturbed mental function is due to an imbalance in the production or metabolism of serotonin. If the role of serotonin (or norepinephrine) in brain function is that of a chemical mediator, especially of subcortical centers, it seems unlikely that the many kinds of mental illnesses could possibly be explained by the single premise of faulty nerve transmission. This seems too easy a solution of the problem. The chemistry of the brain that could account for the specialized functions capable of memory, evaluation, and selection is undoubtedly extraordinarily complicated, far more so than that which is responsible for the specialized functions of other organs, about which we still know relatively little.

The abundance of research with the tranquilizing and hallucinogenic agents and with serotonin may not lead to a profound understanding of schizophrenia, but it may well result in a better comprehension of the integration of the subcortical centers in the brain that regulate the autonomic homeostatic mechanisms. Perhaps it is even more important that research with serotonin and LSD has channeled our thinking concerning brain function and the action of drugs thereon along lines other than those of oxygen consumption, carbohydrate metabolism, the Krebs cycle, and high-energy phosphate formation. These reactions occur in all living organisms, even in the lowest microorganisms. The body is not simply an agglomeration of similar cells, but it is differentiated into organs that have specialized functions, for which specialized biochemical reactions must be responsible. It is probable that subtle biochemical events, peculiar to the brain, will ultimately explain normal brain function and the changes responsible for mental illnesses.

In conclusion, the concept has been developed that a number of mutually antagonistic centers that regulate homeostatic mechanisms in the brain are innervated by serotonergic and adrenergic nerve fibers. It is emphasized that much of the evidence for this thesis is indirect and even meager, and it represents undoubtedly far too simple an answer for a very complicated problem.

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SEROTONIN-LSD INTERACTIONS

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About 5 years ago various workers in my laboratory started to look for drugs that would antagonize the actions of 5-hydroxytryptamine (5-HT, enteramine, serotonin). Many indole compounds depressed the responses of plain muscles, but this effect was generally unspecific, since the effects of other stimulant drugs were also depressed. The action of 5-HT on the rat's uterus was depressed by gramine, however, and this effect was specific because the action of acetylcholine was not depressed, so that this organ was chosen for screening tests. Tests were made with a long series of indole compounds, some of which were very active, and it was eventually found that LSD was more active than any of our synthetic compounds and more active than any of the other ergot derivatives with which we had been working (Fingl and Gaddum, 1953; Gaddum, 1953). It had some effect in a concentration of 10^{-9}, and a given dose of LSD antagonized 20 times its own weight of 5-HT. The effect was specific, since the effects of oxytocin and acetylcholine were not depressed by these concentrations.

Since we already knew that 5-HT was normally present in the brain, this result immediately suggested that the effects of LSD on the brain might be due to interference with the actions of this physiological 5-HT, but it has been difficult to find evidence for or against this theory, and little has been said about it (Gaddum, 1954; Woolley and Shaw, 1954).

In the hands of Hameed the rat's uterus gave very regular results (Gaddum and Hameed, 1954; Gaddum, Hameed, Hathway, and Stephens, 1954), and when the effects were measured and plotted, smooth curves could be drawn showing the relation between the effect and the log dose. When LSD was present in the bath the effects diminished, and the dose of 5-HT was increased. By comparing the effects of these larger doses with the original curves, estimates were made of the dose ratio, which is equal to the ratio between the dose that causes a given effect in the presence of the antagonist and the dose that caused the same effect in the absence of the antagonist. This provided a satisfactory method of measuring the effect of an antagonist and allowed us to show that the effect of LSD, in a concentration of 10^{-8}, continued to increase for at least 45 minutes, during which the dose ratio rose to about 50.

The effects of low concentrations of LSD could always be surmounted by increasing the dose of 5-HT in this way, and reversed by washing the muscle with drug-free solutions. The effects of higher concentrations, however, were insurmountable (in the sense that enormous amounts of 5-HT still had no effect) and irreversible. The tryptamine receptors appeared to have been completely destroyed by these high concentrations.

Ergometrine is also an active antagonist, although earlier experiments failed to detect this effect. Various other ergot alkaloids are also active—more active as antagonists of 5-HT than as antagonists of adrenaline. Dibenamine is a very active antagonist of 5-HT on this tissue.

Experiments with perfused ears from rabbits have generally given results agreeing well with experiments on the rat's uterus. A low concentration of LSD (10^{-9}) antagonizes 5-HT without altering the response to adrenaline. The dose-ratio continues to increase for an hour or more. With a higher concentration (10^{-8}) the effect is much greater and eventually becomes insurmountable and irreversible. This concentration causes slight vasoconstriction, and the response to adrenaline appears to be slightly increased.

Savini (1956) has recently compared the effect of LSD on this preparation with that of brom LSD, whose interesting properties were discovered by Cerletti and Rothlin (1955). This substance was a powerful antagonist for the effects of 5-HT on the blood vessels of the rabbit's ear, but it differed from LSD in various ways. It was about 10 times less active, it never caused vasoconstriction, even in high concentrations, and it acted as an antagonist of adrenaline.

LSD also antagonizes the effects of 5-HT on the blood vessels of perfused lungs and limbs of cats and dogs (Ginzel and Kottegoda, 1953; Gaddum, Hebb, Silver, and Swan, 1953). It inhibits the antidiuretic effect of 5-HT in rats (Erspamer, 1954) and the asthma caused by aerosols of 5-HT in guinea pigs (Herxheimer, 1955).

The guinea pig's ileum behaves differently from these other tissues (Gaddum and Hameed, 1954). The ergot alkaloids are comparatively feeble antagonists of 5-HT on this tissue. Atropine has given discordant results in the hands of different observers. Recent work by Z. Picarelli (unpublished) has shown that there are 2 kinds of tryptamine receptor in this tissue, just as there are 2 kinds of acetylcholine receptor. Type-D receptors, which are thought to be in the muscles, are inactivated by dibenzyline (*N*-benzyl *N*-β phenoxyisopropylchlorethylamine) or LSD. Type-M receptors, which are thought to be in the nervous ganglia, are inactivated by morphine or cocaine. Low concentrations of atropine inactivate type-M receptors only. Higher concentrations inactivate both types.

The evidence for these conclusions depends on 2 kinds of experiments. In the first, morphine (10^{-6}) is present in the bath all the time, so that the M receptors are inactivated. The action of 5-HT on this preparation is antagonized by the drugs that act on the D receptors, such as dibenzyline or LSD, but not by atropine or cocaine. In the second, the intestine has been treated with dibenzyline (10^{-7} for 30 minutes), and the action of 5-HT upon it is antagonized by morphine, cocaine, and atropine, but not by LSD.

These experiments show that the antagonists of 5-HT are not as simple as they might be. When more comparisons have been made between the results obtained by different methods it may be possible to account for various puzzling facts. For example, the substance 1-methylmedmain, which Shaw and Woolley (1953) found to be highly active on arterial rings, has given disappointing results in our experiments on the rat's uterus and has appeared to be much less active than other simple synthetic substances such as 5-benzyloxygramine.

Gaddum and Vogt (1956) have studied the effects of some of these drugs when they are injected into the lateral cerebral ventricle of a cat through a cannula screwed into the skull, as described by Feldberg and Sherwood (1954).

o attempts were made to study minor psychological effects, and the obser- ations were confined to a record of obvious changes in the emotional states the cats. Reserpine or 5-HT made the cats apathetic, lethargic, and un- nterprising, so that they slunk back into their cages and sat still. The actions these substances were similar, but they were not quite the same. After eserpine the pupils were narrow, the nictitating membrane relaxed, and the yes nearly shut. These effects were not produced by 5-HT, but this may ave been because 5-HT did not reach the appropriate part of the brain.

Large doses of LSD, ergometrine, or morphine aroused the cats to a display sham rage, in which they bared their teeth and hissed, but did not bite. This fect was produced even when the cats were lethargic after an injection of HT.

These results suggest antagonistic effects on the centers associated with ousal and with the sympathetic nervous system, but we do not believe that ey are due to the same mechanism as the antagonisms observed on the rat's erus.

Morphine caused arousal in cats depressed after 5-HT, but it had no an- gonistic action on 5-HT in the rat's uterus or the rabbit's ear. This suggests fferent mechanisms, but the position is complicated by the fact that mor- ine does antagonize the effects of 5-HT on type-M receptors in the guinea g's intestine.

β-tetrahydronaphthylamine (β-tet) also causes arousal and has no anti-5-HT tion on the rat's uterus. A. M. Blair (unpublished) has recently tested its tion on the sleeping time of mice anesthetized with hexobarbitone (TABLE . Shore, Silver, and Brodie (1955) showed that 5-HT increases the sleeping me, and that LSD antagonizes this effect. Blair finds that β-tetrahydro- phthylamine has a similar effect. It had no action after hexobarbitone one, but it appeared to diminish the effect of 5-HT in prolonging the sleeping me. These results suggest that the action of LSD in this test is not compar- ble with its anti-5-HT action on the rat's uterus. Again the position is not ite simple, however, since β-tetrahydronaphthylamine has some anti-5-HT tion on the vessels of the perfused rabbit's ear.

Thus these 2 drugs, morphine and β-tetrahydronaphthylamine, both cause citement, and both fail to antagonize 5-HT on the rat's uterus. The oppo- te discrepancy also occurs. The most striking example was discovered by

TABLE 1

Mice. Mean sleeping times (minutes). Doses mg./kg.

Hexobarbitone	150		225	
β-Tetrahydronaphthylamine	0	33	0	33
HT:				
	19.2	21.7	35.2	38.3
	59	24.7	83	40.7
	58	33	94.5	56
	67	—	103	—
	66	—	106	—

TABLE 2

	Anti 5-HT rat's uterus	Schizoid changes	Excitement
LSD	+	+	+
Brom LSD	+	0	0
Ergometrine	+	0	+
Mescaline	0	+	
Morphine	0	0	+
β-Tetrahydronaphthylamine	0		+

Cerletti and Rothlin (1955), who found that brom LSD antagonizes 5-HT i isolated organs, but does not act like LSD in the central nervous system. W have confirmed this conclusion and have added a little to it. Brom LSD an tagonizes 5-HT both in the rat's uterus and the rabbit's ear, but it does nc arouse cats when it is injected into the cerebral ventricle in doses equal to e fective doses of LSD. The synthetic antagonists methylmedmain and 5-ber zyloxygramine also failed to arouse the cats. Various drugs that have bee potent as antagonists of the action of 5-HT on plain muscle have thus faile to cause arousal. This failure may have been due to the impossibility of giv ing adequate doses, but the available evidence does suggest that the causatio of excitement does not depend on anti-5-HT activity (TABLE 2).

Similar conclusions may be drawn from a comparison of the effects of differ ent drugs in causing schizoid changes in man, and in blocking the action c 5-HT on the rat's uterus. Ergometrine, dibenamine, and brom LSD are a active antagonists of 5-HT on the rat's uterus. If ergotamine or dibenamin had caused schizoid changes like those of LSD, this fact would surely hav attracted attention. Brom LSD was inactive when especially tested for suc effects. On the other hand, mescaline, which is said to have effects on th brain not unlike those of LSD, has no anti-5-HT action on the rat's uterus.

It is clear that these observations do not disprove the theory that LSD act on the central nervous system by antagonizing 5-HT, but that they do diminis the value of the existing evidence favoring this theory.

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Discussion of the Paper

C. H. SAWYER (*Department of Anatomy, University of California Medical*
enter, Los Angeles, Calif.): In the summer of 1955 at the University of Cal-
ornia Medical Center, Los Angeles, Calif., C. G. Gunn and I had the pleasure
f working with Gaddum's colleague, Marthe Vogt, on the interaction of sero-
onin and LSD in the brain of the cat. Intraventricular injections of 0.75 mg.
f serotonin or 1 mg. of LSD in the flaxedil-immobilized cat exerted profound
nd reproducible effects on the EEG of subcortical structures. In certain
egions the effects of the 2 drugs were parallel and, when given together, were
dditive. These included a lowering in amplitude of the EEG activity in the
entromedial, anterodorsal, and posterior hypothalamic areas, various basal
alamic nuclei, the midbrain reticular formation, and parts of the internal
apsule. Intermittent bursts of high-amplitude fast activity were observed
the medullary reticular formation following injections of either agent. On
e other hand, LSD increased the amplitude of activity in the amygdala and
duced a high-amplitude 50/second activity in the putamen and globus pal-
dus, while serotonin either decreased the amplitude of the activity in these
reas or slowed it to 10 to 12/second. The LSD enhancement of amygdaloid-
asal ganglia activity lasted roughly as long as does LSD-induced sham rage
the unrestrained cat, while serotonin depression of the same areas had about
e same duration as the lethargic behavior induced by serotonin. The work
ggests that the neural correlates of the effects of LSD and serotonin on be-
avior may be found in these rhinencephalic-basal ganglia areas and their
rojections.

P. GLEES (*Oxford University, Oxford, England*): One wonders whether there
any difference between the serotonin content of the anterior hypothalamus
nd the posterior hypothalamus. We found recently[1] that the effects of large
oses of reserpine could be dramatically reversed by the injection of ampheta-
ine or phenidylate (Ritalin), a new synthetic compound* that has a central
imulating action. A monkey was given 5 mg. of reserpine intramuscularly;
or 3 hours later a parkinsonianlike tremor developed and spontaneous activity

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* CIBA Laboratories, Harsham, Sussex, England.

ceased, but 10 mg. of amphetamine or 20 mg. of phenidylate abolished the severe reserpine syndrome within a few minutes. I feel that this effect could be best attributed to the restoration of the central sympathetic hypothalamic function.

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EVIDENCE FOR THE PARTICIPATION OF SEROTONIN IN MENTAL PROCESSES

By D. W. Woolley and E. N. Shaw

The Rockefeller Institute for Medical Research, New York, N. Y.

The idea that serotonin plays a role in the maintenance of normal mental unctions arose from studies of antimetabolites of this hormone. It was put orward originally by Woolley and Shaw in 1954* and has seemed to be so useul that it may be advisable now to recall the evidence that suggested it, and ome of the subsequent findings that seem further to support it.

Let us first consider what an antimetabolite is. In living creatures there re a variety of important chemical compounds that are essential for normal hysiological functioning. These compounds include such substances as hormones, vitamins, and amino acids. Serotonin is one of these hormones that as a role to play in living processes. One can picture it as causing its effects y combination with specific receptor sites, designed especially to react with t and not with other hormones. The serotonin fits the receptor much as a ey fits a lock, and many features of the hormonal structure are required so hat the serotonin key will function in the receptor lock. If one now makes a ubstance that resembles serotonin in several ways, but differs in a few, one ay have formed an antimetabolite of this hormone. The analog fits the eceptors well enough to combine with them, much as an ill-fitting key can e thrust into a lock, but just as such a related key may not turn the lock, so lso one pictures the substance resembling serotonin as being unable to fulfill he physiological role of serotonin. Furthermore, the presence of this substitute prevents the hormone itself from combining with the receptors, and hus brings about a specific deficiency.

We have used this crude mechanical analogy so that we may visualize the ode of action of these substances. The antimetabolites of serotonin are hemical compounds that resemble this hormone in structure. They bring bout a deficiency of this hormone specifically because they combine with the erotonin receptors by virtue of their structural resemblance to it.

The first pharmacological property of serotonin to be discovered was that t caused smooth muscles to contract. Thus it can be used to cause the muscles of the walls of blood vessels to contract, and possibly for this reason serotonin raises blood pressure in an animal. The uterus and the intestines likewise are made to contract. As Irvine H. Page has already told us elsewhere in this monograph, these are the properties that led to the discovery of serotonin. These properties of serotonin were therefore the first used in efforts to find antimetabolites of it.

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* We regret that we must call attention to an error that recently seems to have gained ground by reiteration; namely, that J. H. Gaddum was the first to discover a possible relationship between serotonin and ental processes. The facts are the following: in April 1954,[1] and in subsequent complete papers published n April 1954[2] and July 1954,[3] we put forward this idea. It was the result of studies, the first of which was published in 1952.[4] The first papers were entitled "A biochemical and pharmacological suggestion about certain ental disorders." Later in 1954, in a paper entitled "Drugs antagonistic to 5-hydroxytryptamine,"[5] Gaddum entioned as a speculation that serotonin might be concerned in mental processes. He based this possibility on is finding that LSD was an antagonist of serotonin in the rat uterus. This finding had appeared as an abstract n the summer of 1953[6] but without any mention of its relationship to the nervous system. Prior to this we had ublished both an abstract[7] and a complete paper[8] in which we showed that ergot alkaloids, of which LSD is one, re antimetabolites of serotonin. In fact, our first findings in this respect were communicated to the *Journal of he American Chemical Society* in July of 1952, and our first paper, which presented a variety of evidence for serotonin in mental processes, was sent to *The Lancet* in the summer of 1953. Because of these facts we feel that we riginated this idea. We also feel that Gaddum arrived at the idea independently, and we believe that he agrees ith us in our appraisal. We regret very much the need to present the matter in this way, but a different picture as often been presented, and the idea seems to be of such importance that we have felt it advisable to set he record straight.

Two *in vitro* tests for antiserotonin activity were devised. The one employed ring-shaped segments of carotid arteries and the other employed the isolated uterus taken from a rat in estrus. In the artery-ring method, the sections of the arteries were suspended in Ringer's solution, and the internal diameters, that is, the diameters of the lumens, were measured with a low-power microscope and a micrometer eyepiece. Enough serotonin was then added to the bath to cause contraction. The amounts needed for this and the relation between dose and response are shown in FIGURE 1. Usually 0.2 γ/cc., an amount that gives a large contraction, was used. To these contracted rings, graded amounts of the analog to be tested were added, and the diameters were again measured. In this way the amount of antimetabolite

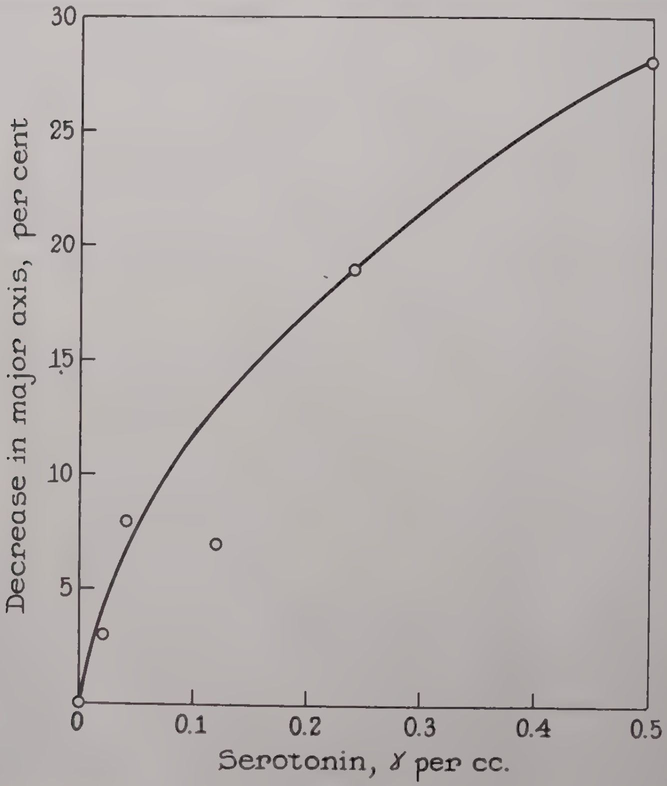


FIGURE 1

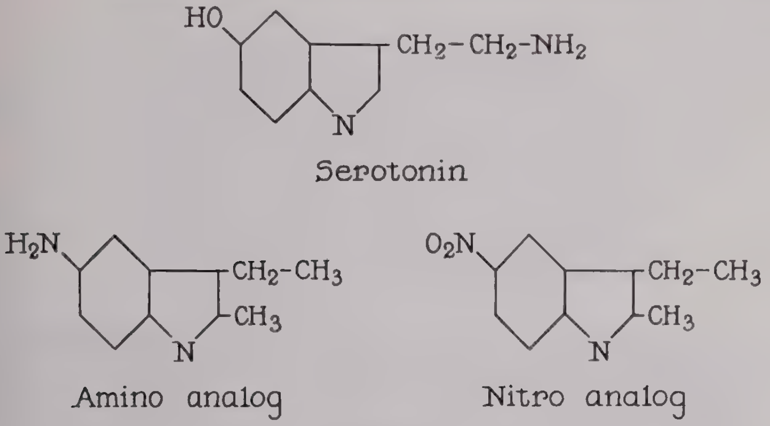


FIGURE 2

needed to cause half-maximal relaxation was estimated. In the uterus method the same general procedure was employed. Thus it was possible to determine whether a compound was an antagonist of serotonin and to measure its potency quantitatively.

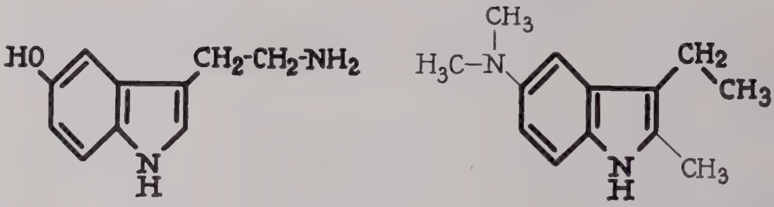
Several structural analogs of serotonin were synthesized and were shown to act as antimetabolites of the hormone in these test systems. The first one was the amino analog shown in FIGURE 2.[4] Some data illustrating its antagonism to serotonin as measured with artery rings are given in TABLE 1. Another early antimetabolite of serotonin that we synthesized was related to this one and was called medmain.[9] Its structure is shown in FIGURE 3. It exhibited very great antagonistic activity, especially in the artery-ring test. In the uterus test it was also active, as can be seen from the tracing in FIGURE 4. Medmain is of interest to us in this discussion because it was one of the first synthetic antimetabolites of serotonin found that caused central effects in animals. In mice it caused convulsive fits suggestive of epileptic seizures.[9]

While working with these synthetic antimetabolites of serotonin the thought

TABLE 1

ANTAGONISM TO SEROTONIN OF 2,3-DIMETHYL-5-AMINOINDOLE MEASURED IN ARTERY RINGS

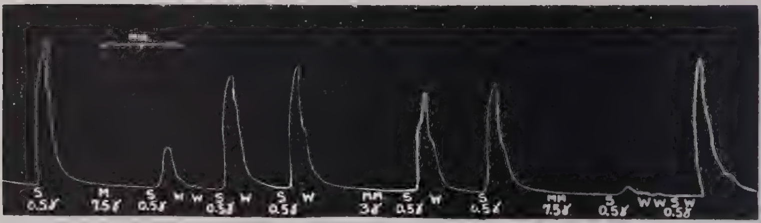
Serotonin	Analog	Decrease in major axis
γ Per cc.	*γ Per cc.*	*Per cent*
0	0	0
0.2	0	18
0.2	0	20
0.2	4	18
0.2	10	9
0.2	20	4
0	20	0



Serotonin

Medmain

Figure 3

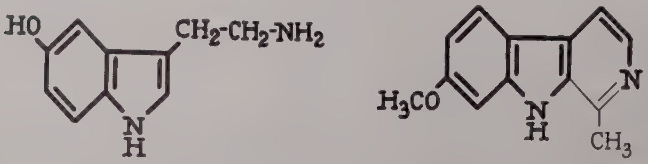


S = serotonin
M = medmain
MM = 1-methylmedmain
W = wash

Figure 4

occurred to us that several classes of naturally occurring alkaloids were structural analogs of serotonin. It was then readily demonstrated that they were competitive antagonists of this hormone. Clearly, then, these alkaloids were naturally occurring antimetabolites of serotonin, and they possibly owed many of their pharmacological effects to this fact. In other words, their actions on animals were partly due to the production of a specific deficiency of this hormone.

The harmala alkaloids, of which harmine is a representative, are one such class of naturally occurring antimetabolites of serotonin. Their structural resemblance is shown in FIGURE 5, and the antagonism to serotonin, as measured with the uterus test, is shown in FIGURE 6.



Serotonin

Harmine

Figure 5

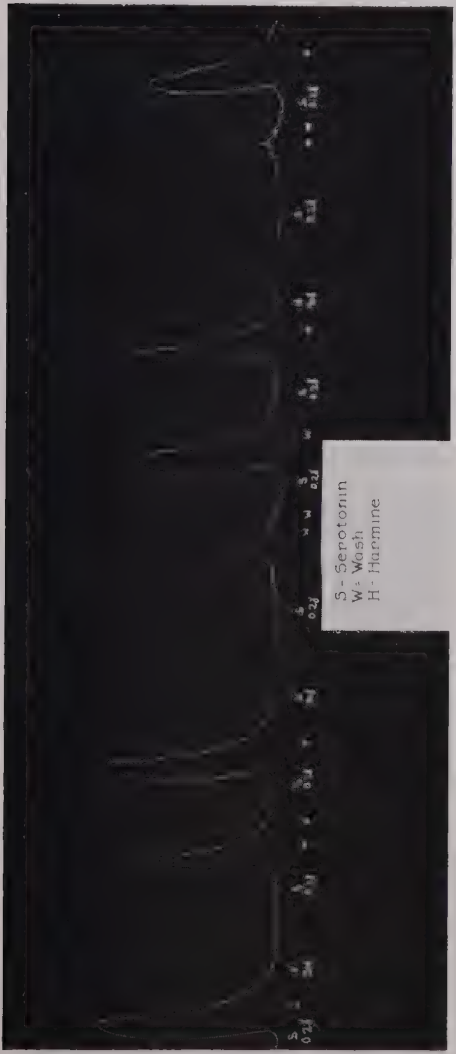
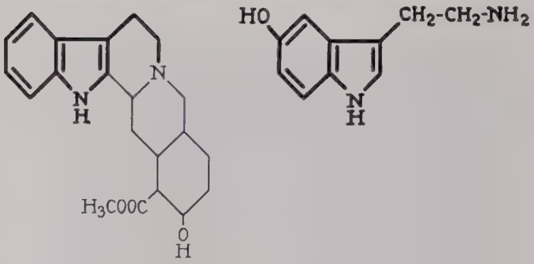


FIGURE 6



Yohimbine Serotonin

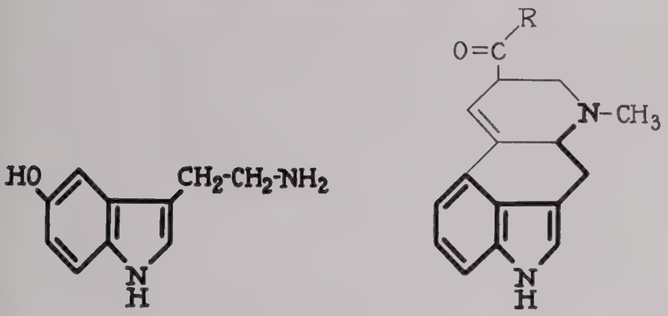
FIGURE 7

Yohimbine (FIGURE 7) is somewhat more complicated in its structure, and hence its resemblance to serotonin is less striking. It can be pictured as merely a harmala alkaloid to which 2 more rings have been added. TABLE 2 shows some of the data that revealed the antagonism between serotonin and yohimbine as seen in the artery-ring test. In this tissue yohimbine is a very powerful antimetabolite of serotonin.

After these demonstrations with yohimbine were published,[7, 8] the chemical structure of reserpine was established, principally by work in the laboratories of CIBA Pharmaceutical Products, Inc., Summit, N. J.[10] Reserpine was shown to be a derivative of yohimbine. One could therefore entertain the hypothesis that reserpine also might be an antiserotonin. Much recent evidence, such as that presented by Shore *et al.* elsewhere in these pages, has now made this seem quite probable. As Shore has told us, reserpine displaces serotonin from the tissues that contain it. This is exactly the mode of action of antimetabolites. Thus, for example, the classical antimetabolite of vitamin B_1, namely pyrithiamine, has been shown to displace the vitamin from animals, and antiacetylcholines have been shown similarly to displace acetylcholine from its receptors. We feel therefore that there is evidence to show that reserpine acts as an antimetabolite of serotonin. Recently Costa[11] has demonstrated directly the antagonism between reserpine and serotonin with the isolated rat uterus, but we must say that this demonstration is not easy,

TABLE 2
ANTAGONISM BETWEEN SEROTONIN AND YOHIMBINE IN SEGMENTS OF SHEEP CAROTID ARTERY

Serotonin	Yohimbine hydrochloride	Contraction in major axis
γ Per cc.	*γ Per cc.*	*Per cent*
0	0	0
0.2	0	20
0.2	0.06	28
0.2	0.2	6
0.2	1.0	4
0	4.0	0
2.0	0	26
2.0	0.2	26



Serotonin

Ergot alkaloid
(When R is diethylamino-,
= lysergic acid diethylamide;
When R is a peptide,
= ergotamine, ergotoxine, etc.)

FIGURE 8

especially when compared to similar demonstrations with yohimbine in the artery-ring test.

The ergot alkaloids are a third class of alkaloids that we have found to be antimetabolites of serotonin. The structural resemblance to the hormone is shown in FIGURE 8. In the ergot alkaloids, the side chain of the hormone has been bent around and fused to the indole ring in the direction opposite to that in the case of the harmala alkaloids, and of yohimbine and reserpine. A new type of analog is thus formed. Several ergot derivatives, such as ergotamine and ergotoxine, have been shown to act as antimetabolites of serotonin in the test systems just described. The most active of these is lysergic acid diethylamide (LSD). Data obtained with this derivative as an antagonist to serotonin in artery rings are shown in TABLE 3.

Our demonstrations that the ergot alkaloids and yohimbine are antimetabolites of serotonin were made early in 1952. We believe that this was the first demonstration of this concept. The findings were first communicated to the

TABLE 3
SEROTONIN VERSUS LSD IN THE CONTRACTION OF CAROTID ARTERY RINGS

Serotonin	LSD	Decrease in diameter of ring
γ *Per cc.*	γ *Per cc.*	*Per cent*
0	0	0
0.2	0	31
0.2	0.03	23
0.2	0.1	15
0.2	0.3	12
0.2	1.0	6
0	10.0	−3

American Chemical Society in July of that year, but the *Journal of the American Chemical Society* declined to publish them. Publication was thus delayed for many months, until they finally appeared in the *Federation Proceedings* the following March (1953), and in the *Journal of Biological Chemistry* that same year, *in extenso*.[7, 8] Gaddum and his associates apparently quite independently hit upon the idea that the ergot alkaloids might be antiserotonins and began to publish their data relative to this in 1953.[6] We believe that these were quite independent discoveries that must have occurred at about the same time. We mention this only to clarify a situation that has confused several authors recently.

Serotonin got into psychiatry because all of the antiserotonins that we have described, both natural and synthetic, cause mental disturbances or other psychiatric effects when given to men. Other compounds that can be shown to act as antagonists to, or to potentiate, serotonin also have been shown to influence the psychiatric state of human beings. Such a compound is hydrazinophthallazine (Apresoline).[12, 13] Some of these compounds, for example, chlorpromazine, can be demonstrated to interfere with serotonin action *in vitro* even though they bear no structural relationship to it and hence cannot be considered antimetabolites of it. These compounds must exert their interference in some other way. The important thing, however, is that they do cause interference. All of this suggests rather strongly that the mental effects of these drugs may arise because they are concerned with serotonin in the brain. On isolated smooth muscles they cause a specific deficiency of serotonin in these tissues. Perhaps they do the same thing in the brain by combining with the serotonin receptors there and thus excluding the hormone from its functioning site. This is the simplest statement of the case. Some elaborations of it that are necessary will be mentioned later in this article.

If this idea is correct, the presence of serotonin is required in the brain and a demonstration is needed to show that serotonin affects the functions of the brain. Fortunately the presence of this compound in the brains of mammals and in the nerves of some invertebrates has been detected by investigators in several laboratories. Already in this monograph, Page, Gaddum, and Welsh have all told us of such demonstrations.

The next problem was to determine whether or not serotonin had a function in the nervous system. You will recall that all of the tests for antiserotonin activity were carried out on smooth muscles. Are we justified in applying the findings in muscles to interpret what happens in the brain? A most fortunate collaboration[11] with Margaret Murray and Helena Benitez of the College of Physicians and Surgeons, Columbia University, New York, N. Y., has provided some clues in this connection.

The brain contains some cells, the oligodendroglia, that are characterized by a pulsating, rhythmical movement. These cells slowly contract and expand. We considered the possibility that the contractions might be caused by an action of serotonin on the elements in these cells similar to the serotonin receptors in smooth muscles. Murray and Benitez have studied these cells in tissue cultures and have observed that serotonin causes them to contract strongly. Furthermore, by addition of an antimetabolite of serotonin, this

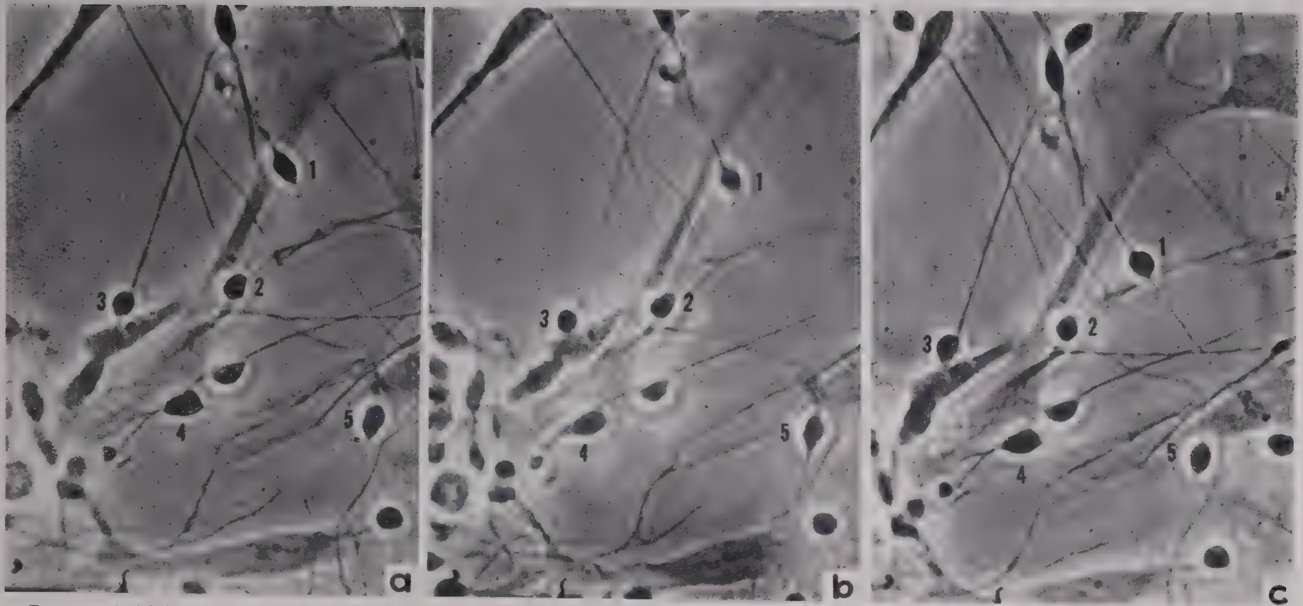


FIGURE 9. Serial photographs of a control culture of fetal human oligodendroglia, showing normally pulsating cells. Note the changes in the outline of the numbered cells. In FIGURE 9*b* cell No. 1 contracted during an exposure time of 2 minutes, producing the recorded blurred outline.

strong contraction can be overcome, and the cells can be restored to normal pulsations. Fetal human brain tissue has been used for these experiments, and such antiserotonins as medmain and LSD have been tested.

To show the effects most clearly a time-lapse movie is required. Since this is not practical for this monograph we have illustrated the salient features with a few photographs. FIGURE 9 shows a group of oligodendroglia. Because some cells were in a state of contraction and others were relaxed when this picture was taken, it is necessary to follow the actions of individual cells in order to see what happens. For this purpose, certain cells in the photographs have been marked. Thus cell No. 1, in the 3 exposures shown, can be seen relaxed, then in the process of contraction, and finally contracted. The fuzziness of the outline in the middle picture is a result of changes in the cell outline during the exposure of the film. FIGURE 10 shows the fully developed contraction as seen 30 minutes after application of the serotonin (5 γ/cc.). The effect is greatest at this time. The cells remain in this contracted state for about 1 hour, when they slowly resume normal pulsations. After application of medmain (50 γ/cc.) these contracted cells were restored to normal.

Thus in these elements of the human brain one can demonstrate serotonin action and its reversal by an antiserotonin, just as one can with smooth-muscle preparations. This contraction caused by serotonin is not a property

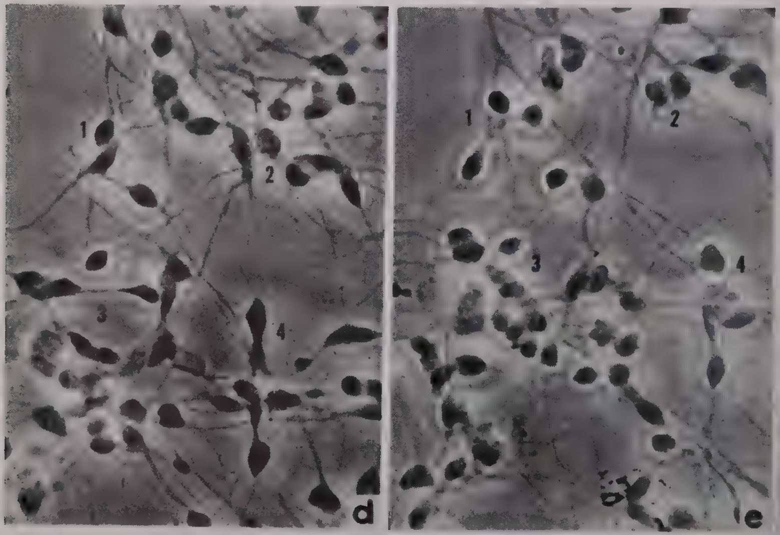


FIGURE 10. Effects of serotonin at 5 γ/ml. on fetal human oligodendroglia. FIGURE 10*d* shows the culture before the administration of the drug, and *e* shows the same culture 36 minutes after serotonin was introduced into the medium. Note particularly the behavior of the numbered cells. Following exposure to serotonin the individual cells enter into a tetanic contraction, and this may result in the drawing together of groups of cells, as shown by the groups around the numbered cells.

shared by all kinds of brain cells. For example, serotonin did not cause astrocytes to contract.

With LSD applied to oligodendroglial cells the effects were more complicated than with medmain. LSD first caused a relaxation and vacuolization of the cells that eventually subsided and was succeeded by a strong contraction. Serotonin augmented this contracting effect, so that in this respect LSD was serotoninlike rather than antagonistic. However, the relaxation and vacuolization that were the initial effects of LSD were prevented by serotonin in a concentration too low to cause discernible effects by itself. There was therefore an antagonism to be seen as well as a serotoninlike effect.

Although these experiments are only in their early phases, we feel that they indicate that it is possible to demonstrate a visible action of serotonin on certain elements of the brain and that they lend support to the idea that what has been found in muscles with respect to antiserotonins may also have importance in the brain. A summary of the findings with the cultures of oligodendroglia is presented in TABLE 4. A more detailed description may be found in the published accounts of this work.[14]

The real test of our hypothesis that antimetabolites of serotonin cause psychiatric disturbances by interfering with the action of serotonin in the brain would be to determine whether serotonin would overcome the mental effects of the drugs. We have endeavored to do this, and we have had some success.

TABLE 4

SEROTONIN AND ANALOGS: EFFECTS ON OLIGODENDROGLIA

Compound	Concentration per ml.	Results
Serotonin	2 γ	Ineffective (normal pulsation)
Serotonin	5 γ	Prolonged contraction for 60–90 minutes
Medmain	100 γ	(Toxic) contraction starting after 3 hours
Medmain	200 γ	Immediate contraction (toxic)
Serotonin + medmain simultaneously	5 + 50 γ	Normal pulsation
Serotonin + medmain after 33 minutes	5 + 50 γ	Releases serotonin-contracted cells
1-Methyl-medmain	5 γ	Ineffective (normal pulsation)
1-Methyl-medmain	50 γ	Ineffective (normal pulsation)
1-Methyl-medmain	200 γ	(Toxic)
Serotonin + 1-methyl-medmain simultaneously	5 + 50 γ	Normal pulsation
Serotonin + 1-methyl-medmain after 30 minutes	5 + 50 γ	Releases serotonin-contracted cells
LSD	5 γ	Relaxation → contraction → pulsation
LSD	100 γ	Above course prolonged (toxic borderline)
Serotonin + LSD simultaneously	5 + 50 γ	Increases contraction induced by serotonin
Serotonin + LSD after 33 minutes	5 + 50 γ	Increases contraction induced by serotonin
Serotonin + LSD after 52 minutes	5 + 5 γ	Increases contraction induced by serotonin
Serotonin + LSD after 35 minutes	2 + 20 γ	Normal pulsation
Control (balanced saline)		Normal pulsation

The first difficulty we encountered was that of getting serotonin into the brain Peripheral injection failed, probably because of the rapid destruction by amine oxidase and possibly because of difficulty in passing the blood-brain barrier. In any event, injection of large doses of serotonin into mice, followed by analysis of the brain, showed that no detectable amount had lodged there. Subsequent investigators have had similar experiences.[15] Consequently one must devise a method of getting the hormone into the brain and to its proper sites in that organ, and a method of keeping it there, safe from destruction.

You can appreciate the fact that a serious problem now arises. One should use human subjects for experiments on mental disturbances, because it is only a human who can tell you if he is experiencing a mental disturbance. One cannot inject serotonin and its relatives directly into the brains of human beings, however. The risks to health are too great. One must therefore give up any thought of human trials and must try to devise some method of getting the needed information from laboratory animals.

To do this we have observed the behavior of animals treated with serotonin analogs and have attempted to interpret this behavior in terms of natural responses made by a normal animal to specific situations. In this way we have attempted to gain some insight into the thoughts of the animal. This is an uncertain pursuit, but let us examine what we have found.

When a mouse is given LSD intraperitoneally a characteristic train of behavior results.[16] The mouse becomes agitated and looks rapidly from side to side. It holds itself close to the ground and very soon begins to spread its legs and its toes and to push backward with its forelegs. Soon it begins to walk backward, and it will push strongly against any object that prevents it from doing so. The ears become very red, the hair stands on end, and vigorous tremors may ensue.

Many of these signs can be observed in untreated mice placed in the following situation. The normal animal is placed on a large glass plate so that it faces downhill as one edge of the plate is raised. The mouse thus faces the prospect of sliding downhill, and indeed it does this as the slope becomes steeper. Just before it begins to slip the mouse looks agitatedly from side to side, crouches low, spreads its legs and its toes, and pushes backward with its forelegs, apparently in an effort to prevent the sliding. Comparing this behavior with that of the LSD-treated mice, we have concluded that the mice given LSD and kept on a level surface have a feeling of sliding downhill. In fact, if one tilts the surface on which they stand so that they face uphill, the pushing and walking backward cease. The reddening of the ears, the horripilation, and the tremors are not seen in the normal animals on the inclined plane, and thus these must represent additional effects of LSD.

If we entertain the postulate that the mouse treated with LSD pushes and walks backward because of a hallucination, we should be able to prevent or overcome this effect by injecting serotonin into the brain. Table 5 shows what has been found. Serotonin alone, injected into the brain, will not prevent the behavioral change. If a long-acting cholinergic drug is given with it, however, then about half of the animals can be protected partially or fully.

There are many considerations to be taken into account in the interpretation

TABLE 5
ANTAGONISM BETWEEN LSD-25 AND SEROTONIN PLUS CHOLINERGIC DRUGS IN THE ABNORMAL BEHAVIOR OF MICE*

I. C. Injection	No. of mice	No. pushing but not walking backward	No. walking backward
Saline	19	0	19
Serotonin 20γ	5	0	5
Carbamylcholine 0.2γ	13	0	13
Serotonin 20γ + carbamylcholine 0.2γ	23	3	14
Physostigmine 1.0γ	5	1	4
Serotonin 20γ + physostigmine 1.0γ	5	3	2

* All mice were given 100 γ of LSD-25 each intraperitoneally.

of experiments of this sort, but we shall not go into a detailed discussion here. Some of these points have been covered in another published account.[16] In any event, some of the behavioral changes induced in mice by LSD can be prevented with serotonin. In this situation, then, one can find evidence that the mental disturbance caused by the drug is the result of an interference with serotonin.

If all that is required to cause mental disturbances is an antagonist of serotonin, then all antimetabolites of serotonin should make mice walk backward or should elicit hallucinations in men. There is clearly something more than this involved because many antiserotonins have been found to cause mental disturbances, but the sensations are not identical with each, and some antiserotonins have not been observed to cause any effect of this sort. Does this, then, invalidate the basic hypothesis? For example, does the failure of ergotamine to cause mental effects similar to those caused by LSD, even though both compounds are antiserotonins on artery rings, mean that the mental effects of LSD are not the result of its antagonism to serotonin? Or does the diminished hallucinogenic action of bromo-LSD, as described by Rothlin elsewhere in this monograph, similarly invalidate the idea? We do not feel that this is the case.

Although ergotamine given peripherally will not induce behavioral changes such as those caused by LSD, it will produce these changes if it is injected into the brain. Thus we have found that intracerebral administration of ergotamine to mice will cause them to walk backward in a manner similar to that of LSD mice.

Recently we have been able to synthesize an antiserotonin that is unusually active in overcoming the pressor action of serotonin. This compound has proved capable of reducing the high blood pressure of human patients suffering from essential hypertension and has thus validated the original postulate put forward with respect to this disease 4 years ago.[4] This new and potent antiserotonin is 1-benzyl-2,5-dimethylserotonin (FIGURE 11). The first antiserotonin tested in the treatment of hypertension, namely, the nitroindole shown in FIGURE 2, had proved to be unusable because of the mental depression that it caused. This finding was made in collaboration with R. Wilkins and his co-workers*. Before the clinical trials we had observed even in mice that long

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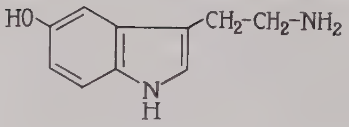
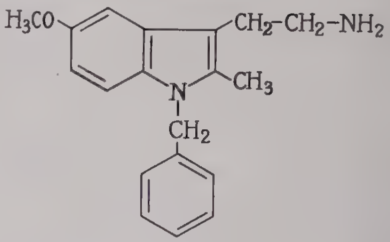
* Unpublished data.



Serotonin



1-Benzyl-2,5-dimethyl-serotonin

Benzyl analog of serotonin (BAS)

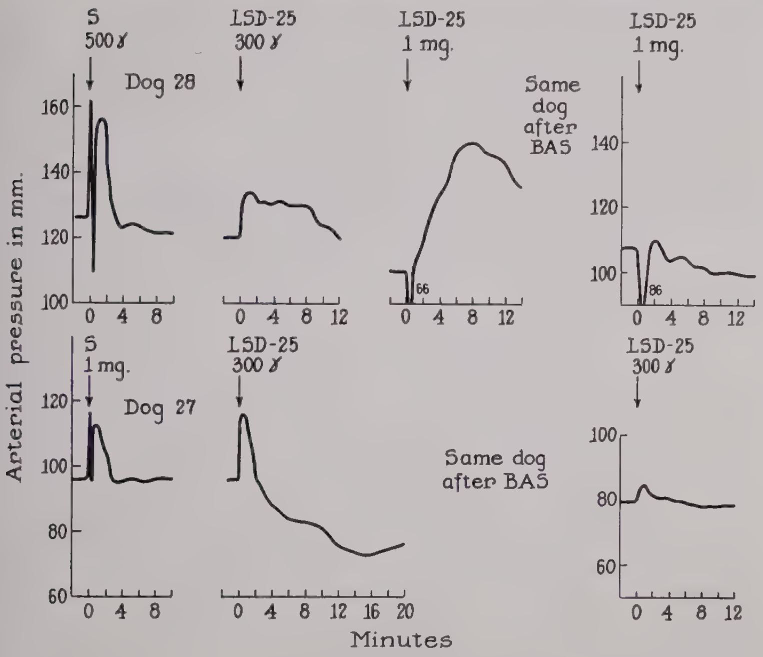
FIGURE 11

treatment with this nitroindole altered the personality of some so that they became unusually savage and would bite anyone who attempted to feed them. These were vague symptoms, and they were seen in only a small percentage of the animals, but the human experience substantiated them. When potent hallucinogenic compounds such as harmine and LSD were shown to be antiserotonins, the general reaction was that all antiserotonins would probably disturb the mind. This has proved to be not necessarily true. The benzyl analog of serotonin shown in FIGURE 11, which may be abbreviated as BAS, has not given trouble of this sort in human patients, according to the findings of Hollander *et al.*[17]

Similarly, this potent antiserotonin will not cause mice to behave as they do when given LSD. The only behavioral change that has been detected in these animals is a slight drowsiness.

This compound was designed in the belief that it would not unduly penetrate to and affect the brain. One can test the idea that it is a failure of penetration that accounts for the failure to cause behavior changes merely by introducing BAS directly into the brain. When this is done with mice they do exhibit some of the signs caused by LSD. Thus, if the dose is properly adjusted (50 γ per mouse into the brain) the pushing and walking backward can be produced. In addition the animals tend to rise up on their hind legs, and even to fall over backward. None of these signs has ever been seen in mice treated peripherally with large amounts of this compound. Experiments of this sort might suggest that some antimetabolites of serotonin fail to cause mental disturbances because they fail to reach the proper sites in the animal. Quite probably this is not the only reason.

A final aspect of this general idea must be mentioned. The foregoing discussion has revolved around the concept of a deficiency of serotonin as the cause of the hallucinations called forth by harmine, LSD, yohimbine, and other relatives of the hormone. There is also the possibility, however, that these compounds penetrate the brain and act on its receptors just as serotonin does. This is more than an academic possibility. We must remember that LSD, when tested on oligodendroglia from the brain, caused contractions just



Fifty mg. of BAS given i.v. 1 hour before last LSD-25 injection

FIGURE 12

as did serotonin, and that the hormone did not reverse this effect. We have also found that in other test systems LSD has an effect similar to that of serotonin.[15] For example, it acts as serotonin does in the stimulation of the heartbeat of the clam *Venus mercenaria*. LSD also increases the blood pressure of dogs just as serotonin does and, in this effect, the LSD is antagonized by BAS. This specific antagonism therefore increases the evidence that the pressor effect actually is serotoninlike (FIGURE 12).

Some of the other analogs of serotonin that affect the central nervous system likewise can be found to have a serotoninlike component of their actions. Thus medmain, when tested on the uterus or on oligodendroglia, exhibits a serotoninlike action if the concentration used is high enough. On the heart of the clam Welsh found the nitroindole of FIGURE 2 to act as serotonin does, whereas we have found it to be only antiserotonin in the blood-pressure test with dogs. Thus it is clear that the same compound can have both proaction and antiaction, depending upon the test object and the concentrations used.

From considerations such as these it seems that it is not possible to decide between the alternatives of deficiency or excess of serotonin as the reason why these relatives of it bring about their effects on the brain. Both possibilities must be kept in mind. A compound such as LSD might combine with a serotonin receptor and fit well enough to perform the functions of the hormone itself (serotoninlike action). With other serotonin receptors, such as those in artery rings, the LSD combines, but the fit is too imperfect to allow functioning as serotonin. The LSD then excludes the serotonin from the same site and acts as an antagonist.

Despite this uncertainty as to whether the relatives of serotonin are acting as the hormone does or against it, the evidence seems rather clear that the maintenance of normally functioning serotonin levels in the brain is needed for normal mental processes. The original evidence for this concept, which was published in 1954,[1, 2, 3] has now been strengthened by a variety of findings in several laboratories.

What are the functions of serotonin in the brain that might account for its relationship to mental processes? We believe that the experiments with oligodendroglial cells suggest one possible explanation. The brain is poorly vascularized in comparison with organs such as the kidney, and this has led to the belief that the oligodendroglia are little stirring devices designed to facilitate circulation of extravascular fluid. In this fashion the passage of oxygen and of food (and of waste products in the opposite direction) may be hastened. An excess of serotonin could stop these stirrers by causing a tetanic contraction such as one observes in the tissue cultures. Furthermore, if the pulsations of these cells arise from the rhythmical production therein of serotonin, followed by the destruction of the compound (to permit relaxation after each contraction), then a failure to produce enough serotonin, that is, a metabolic deficiency of serotonin, could slow down the stirring action. An antimetabolite of serotonin, acting in the classical fashion, would accomplish this same end of bringing about a deficiency. If the stirring action is interfered with, the normal supply of nutrients and the removal of waste is impeded. It is well known that hallucinations and central effects such as convulsions are caused by anoxia. Hallucinations closely resembling the symptoms of mental disease may be produced in mentally well people by hypoglycemic episodes. One of the authors of this paper (D.W.W.) has experienced several of these brought about by the action of insulin. They probably represent a reaction to a reduction in the fuel supply to the brain cells. Such a reduction might also be the result of a slowing of the normal pulsations of the oligodendroglial cells. Quite probably the only function of serotonin in the brain is not to cause contractions of these glial cells, but we should not overlook the strong possibility that this is one function.

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Discussion of the Paper

M. M. Rapport (*Sloan-Kettering Institute for Cancer Research, New York, N. Y.*): In connection with Woolley and Shaw's presentation of the action of antimetabolites of serotonin, it may be worthwhile to emphasize that several receptors or binding sites of a heterogeneous nature must be considered in attempting to understand the pharmacological and physiological actions of a chemical mediator and its inhibitors. There are the binding sites involved in the formation, storage, and chemical degradation of the substance in addition to the receptors in the target organ, and the complexity of the observed results with some of the drugs we have been considering must certainly be ascribed to the fact that a given drug acts at more than a single locus. The relative effectiveness at each locus might be expected to show considerable variation. For example, while we may ascribe to reserpine a principal role in affecting the storage sites of serotonin, it cannot be denied that reserpine also has an action on the cells of the target organ. We are completely ignorant, however, of its relative potency at the 2 loci, as well as of its relative effectiveness on fundamentally different, serotonin-responsive cells such as those of muscle and nerve.

Most binding or receptor sites are considered to be protein in nature and, to this extent, they escape our present capacity for understanding. I have been thinking, however, of some evidence originating in the isolation of 5-hydroxytryptamine from natural sources that has relevance to the problem of binding sites and offers us an opportunity for appreciating a detailed mechanism. Erspamer noted in 1940 that the pharmacological activity of an acetone extract of rabbit stomach was increased after heating with alkali. This behavior is in marked contrast to the instability of serotonin in aqueous solutions or in serum, and the lability of the substance to almost any kind of treatment was one of the major obstacles in its isolation. Erspamer called this pharmacologically inactive form "enteramine I" and, in 1951, from color reactions on a paper chromatogram, he demonstrated its probable close chemical relationship to active enteramine. He then found that the inactive form could be activated by simple treatment with phosphate buffer. The inactive form is not attacked by amine oxidase and must therefore be bound at the ethylamine side chain, in particular through the amino group. Since it gives the 5-hydroxyindole color reactions, but is stabilized against interactions that normally inactivate the sensitive hydroxyindoles, it would appear that there is a second point of attachment, possibly via the indole nitrogen. These bonds are weak, since they can be disrupted so readily, and they are probably of the ionic- and hydrogen-bond type rather than covalent.

Serotonin shows a very strong tendency to form complexes, and these may play a part in its biological pattern of activity. While the chemist invokes complex formation almost as frequently as the biologist calls forth permeability to disguise his ignorance, the phenomenon is still very real. The infrared absorption spectrum of serotonin creatinine sulfate does not show the band characteristic of the carbonyl group of creatinine, but instead a doublet appears, indicating that in this weak complex a new chemical bond is present. In the procedure for isolating serotonin from serum there is a step in which lipid substances are removed by extraction with chloroform without extracting any of the serotonin. On occasion, almost all of the active substance appeared in the chloroform phase, a property that could be reversed by simple dilution. In a later step serotonin was quantitatively precipitated in combination with ammonium diliturate. In this form it was completely stable, and the diliturate salt could be recrystallized from water indefinitely without appreciable loss of activity. These properties again indicate the capacity of serotonin to form stable complexes. Some of the described properties recall older studies of the binding of epinephrine and noradrenaline by lecithin that have been reported from time to time. I think it might be quite rewarding to investigate the nature of Erspamer's "enteramine I" and the specificity of phospholipid binding in order to gain some more detailed information concerning nonprotein binding sites of serotonin. It might equally contribute to our subsequent picture of protein receptors.

L. C. Clark, Jr. (*Fels Research Institute, Yellow Springs, Ohio*): I have some evidence that agrees with the concept that continuous pulsations occur in the finer structures of the brain. When platinum, silver, gold, or platinum black polarographic cathodes, maintained at 0.6 v. with respect to a subcutaneously implanted Ag-AgCl electrode, are *chronically* implanted in the brains of cats and other animals, one is able to monitor oxygen availability continuously for months. In fact, I have 1 animal with cathodes implanted for over a year and a half. After a short healing period varying between several hours and several days, waves or cycles of 8 to 12 per minute occur in the oxygen current. These oxygen-availability waves are not related to respiration rate or heart rate. They are nearly eliminated by 30-per cent carbon-dioxide inhalation and by hypoxia. They show more complex variations with some of the drugs discussed in papers included in this monograph. I believe they reflect the mechanical stirring of oxygen-containing fluid at the electrode surface, brought about by the cyclic narrowing and widening of blood capillaries and, possibly, by the concerted rhythmic movement of the glial cells.

D. B. Tyler (*Department of Pharmacology, School of Tropical Medicine, University of Puerto Rico, San Juan, Puerto Rico*): I question whether the abnormal behavior produced by LSD in mice (a reaction similar to that shown by normal mice sliding downhill) can be properly characterized as a hallucination. Is it not likely that you may be dealing with a purely abnormal neurological reaction rather than a behavioral or psychic reaction? From the description of the response these animals show to LSD it appears that they may be undergoing a sensation of falling brought on by a disturbance of the nonauditory parts of the labyrinth at the level of the semicircular canals, the otolith organs, or at some point along the nervous connections of the labyrinth with other centers in the brain and the cerebellum. If such is the case the phenomenon is not psychic, but neurological. It is of interest here that chlorpromazine, which is known to have many effects opposite to LSD, is effective in combating the effects of abnormal stimulation of the labyrinthine apparatus, as found in Ménière's syndrome, or as it is brought about by motion.

LYSERGIC ACID DIETHYLAMIDE AND RELATED SUBSTANCES

By E. Rothlin
Pharmacological Laboratory, Sandoz, Ltd., Basle, Switzerland

Ergot has a remarkable history. Man first made contact with it as a violent poison when the consumption of rye infected with ergot led to the severe symptoms of ergotism. How this poison came to be used as a remedy is not quite clear. Formal medicine was opposed to the therapeutic use of ergot, but midwives long ago availed themselves of it for promoting childbirth and for arresting postpartum hemorrhage. Starting with this knowledge, the American physician Stearns[1] published in 1808 the first scientific paper recommending ergot for these purposes. His publication aroused interest in the scientific study of ergot and its therapeutic applications, not only in America but also in Europe. Advances in chemistry and, especially, improvements in preparative techniques, have made it possible to isolate a series of substances, some of which occur elsewhere in nature, the biogenic amines for example, while others, the characteristic alkaloids, are specific to ergot. Until 1925, the use of these alkaloids in medicine was restricted to the fields of obstetrics and gynecology.

A second phase in the history of ergot began when we showed that the sympathicolytic properties of ergot, first described by Dale,[2] had a therapeutic application. Following the clinical trials that we instigated, first ergotamine alone and then the combination of ergotamine with belladonna alkaloids and phenobarbital were added to the therapeutic *armamentarium*.[3] Special interest was aroused by the use of ergotamine in migraine.[4]

The most recent and third phase in the history of ergot followed our discovery of the *central* actions of ergotamine. In 1923, the inhibition of the depressor reflexes was described;[5] in 1934, we were able to show that ergotamine potentiates the action of phenobarbital without itself possessing a hypnotic action; and in 1944, we demonstrated the central action of the hydrogenated alkaloids of the ergotoxine group.[6a, 6b]

The increased interest that followed this extension of the field of therapeutic indications also stimulated interest in the chemistry of ergot. Stoll and his associates[7] succeeded in carrying out a partial synthesis of ergonovine (ergometrine) and, as far back as 1938,[8] a rather closely related derivative, the diethylamide of lysergic acid (LSD), was synthesized by these workers. Like ergonovine, this compound proved to have a pronounced action on the uterus and the vagina when tested in the rabbit. The remarkable psychic effects of LSD were discovered by chance in 1943 when A. Hofmann, a chemist engaged in the preparation of this compound, inadvertently ingested a minute quantity of it.

In experiments on ourselves, my collaborators and I were able to confirm Hofmann's observation, and W. A. Stoll[9] undertook the first systematic psychiatric investigation of this "phantasticum," as he called it.

My object here is to furnish an account of the pharmacological characteristics of LSD and some of its related compounds.

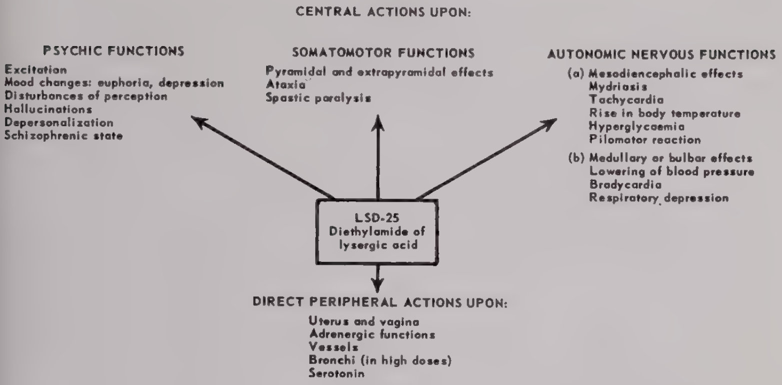

FIGURE 1

FIGURE 1 presents a summary of the complex actions of LSD. Basically, 2 different types of actions may be distinguished: direct peripheral actions and central actions.

Direct Peripheral Actions

These include the actions on the uterus, vagina, blood vessels, and muscles. In addition, LSD exerts a weak adrenergic blocking action and, most important of all, a pronounced antagonism to serotonin, an effect first described by Gaddum.[10, 11]

Central Actions

These actions may be subdivided to show the effects of LSD on 3 groups of functions:

(1) *Autonomic effects.* These effects are brought about by stimulation of the mesencephalon and the medullary centers, such as mydriasis, rise in body temperature, hyperglycemia, piloerection, and inhibition of depressor reflexes.

(2) *Motor effects.* These effects are both pyramidal and extrapyramidal in nature. They lead to ataxia and, especially, to spastic paresis.

(3) *Psychic effects.* The psychic changes are known to vary greatly from one individual to another, and a remarkable feature is the long period of latency. Whereas the autonomic effects set in approximately 20 minutes after administration of the drug, the typical psychic changes do not appear for a period of from 40 to 60 minutes, and they reach their maxima only after 1 to 2 hours. These effects take the form of changes of mood, either in the direction of euphoria or of depression. Particularly characteristic are optic hallucinations, sense of depersonalization, and schizoid states. The entire pattern of mental changes resembles that produced by mescaline, although about 5000 times as much of the latter substance is needed. It may be mentioned here that certain of the autonomic effects, for example an increase in body temperature,

TABLE 1

INHIBITION OF SEROTONIN BY VARIOUS AMIDES OF LYSERGIC ACID

1. LSD and its isomers	
d-Lysergic acid diethylamide (=LSD-25)	very active (standard)
l-Lysergic acid diethylamide *d*+*l*-Isolysergic acid diethylamide	practically inactive, that is, more than 100 times weaker
2. Derivatives of LSD obtained by saturation of the double bond (C_9 to C_{10})	
Dihydro-*d*-lysergic acid diethylamide	1.6 times weaker
Lumi-*d*-lysergic acid diethylamide	practically inactive
3. Substituted derivatives of LSD	
d-1-Acetyl-LSD	2 times stronger
d-2-Brom-LSD (= BOL 148)	1.5 times stronger
d-2-Iodo-LSD	2 times weaker
d-1-Oxy-methyl-LSD	1.5 times weaker
4. Monosubstituted amides of *d*-lysergic acid*	
Monomethylamide of *d*-lysergic acid	15.5 times weaker
Monoethylamide of *d*-lysergic acid	8.5 times weaker
Monoisopropylamide of *d*-lysergic acid	5.0 times weaker
Monopropylamide of *d*-lysergic acid	2.5 times weaker
Monobutylamide of *d*-lysergic acid	1.5 times weaker
5. Disubstituted amides of *d*-lysergic acid*	
Dimethylamide of *d*-lysergic acid	5 times weaker
Diethylamide of *d*-lysergic acid (= LSD-25)	= standard
Di-isopropylamide of *d*-lysergic acid	4 times weaker
Dibutylamide of *d*-lysergic acid	3 times weaker

* Preliminary results.

can be produced in the rabbit with doses not higher than those that cause psychic changes, that is, with approximately 0.5 to 1 μg./kg.

Of the various effects produced by LSD, the psychic effects in man have so far attracted the greatest attention. Not only has LSD been employed as a therapeutic agent in psychiatry, it has also been used to help in the elucidation of a number of theoretical questions that are of interest to the biochemist and the pharmacologist, as well as to the psychiatrist. This research work was given considerable impetus by the detection of 5-hydroxytryptamine (serotonin, enteramine) in the brain (Page,[12] Gaddum[13]) and by the demonstration of the important part played by this substance in cerebral metabolism. As already mentioned, Gaddum[11] demonstrated that LSD exerts a pronounced antagonism toward serotonin.

In the meantime, the chemists engaged in work on the synthesis of these compounds had not been idle. Stoll and his associates[14] provided us with a whole series of related compounds of *d*-lysergic acid for testing.

Thus we had the opportunity of comparing these compounds with LSD. In TABLE 1 are summarized the data concerning the antagonism of these substances to serotonin. We consider these results preliminary because we are not always certain in dealing with specific serotonin antagonism. A reliable analysis of serotonin antagonism requires consideration of the following criteria: (1) the extent of the latency period for maximum action; (2) the reversibility of this effect; and (3) the absence of inhibitory action toward acetylcholine, epinephrine, and histamine at levels of concentration that exhibit serotonin antagonism.

It is remarkable, if not altogether surprising, that of the diethylamides of the 4 known isomers of lysergic or isolysergic acid, only *d*-lysergic acid diethylamide is active (first group of TABLE 1). The other 3 isomers are more than 100 times less active as antagonists of serotonin than is the first, and they do not exert a competitive effect toward LSD.

In the second group of compounds we have those derivatives in which the double bond between C_9 and C_{10} has been saturated either by the addition of hydrogen as in dihydro-*d*-lysergic acid diethylamide or by the addition of water to give the so-called lumi-LSD. The dihydro compound is 1.6 times less effective than LSD in inhibiting serotonin, and lumi-LSD is as much as 100 times less active.

In the third group we have the derivatives in which the lysergic-acid nucleus is substituted. These derivatives include *d*-1-acetyl LSD, *d*-2-brom-LSD, *d*-2-iodo-LSD, and *d*-1-hydroxymethyl LSD. The behavior of these derivatives is noteworthy because substitution of the lysergic acid nucleus yields compounds, such as acetyl-LSD and brom-LSD, which are 1.5 to 2 times as powerful as LSD in inhibiting serotonin, whereas all the derivatives in the other groups mentioned in this TABLE are weaker antagonists. Strangely enough, iodo-LSD is 1.8 to 2 times weaker than brom-LSD in inhibiting serotonin, and the hydroxymethyl derivative shows a similar behavior.

The fourth group comprises compounds having a single substituent at the amide nitrogen. These compounds form a homologous series in which the substituent is a methyl, ethyl, isopropyl, propyl, or butyl group. All these compounds are weaker antagonists of serotonin than is LSD, the comparative igures being in the same sequence: 15.5., 8.5, 5, 2.5, and 1.5 times weaker than LSD. We see here a significant increase of action with the length of the side chain.

Finally, we have the disubstituted amides of *d*-lysergic acid: the dimethyl, diethyl, di-isopropyl, and dibutyl amides. They are 3 to 5 times weaker than LSD in their antagonism toward serotonin.

The most important of these compounds is undoubtedly 2-brom-LSD. TABLE 2 shows a comparison between the effects of LSD and brom-LSD on a

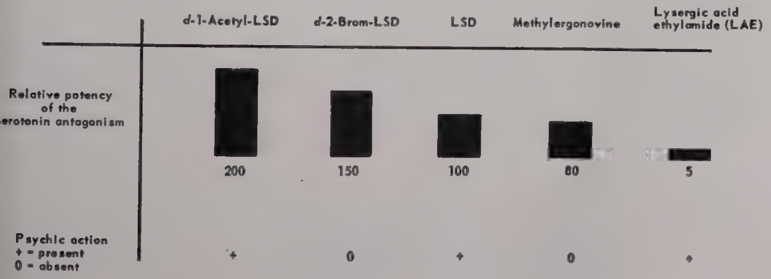

FIGURE 2

variety of functions. It will be seen that although the 2 substances agree in certain properties there are fundamental differences between them. Brom-LSD is completely devoid of action on the psyche. The action of this substance is mainly sedative in character, and central effects on autonomic functions are absent. If anything, however, brom-LSD is at least as powerful an antagonist of serotonin as is LSD.

As already mentioned, it is known that the serotonin antagonism of the various derivatives of *d*-lysergic acid is not of the same intensity in all functions. We have carried out a comparison among the serotonin-inhibiting effects of the 3 most interesting derivatives, LSD, acetyl-LSD, and brom-LSD, on various functions *in vitro* and *in vivo*. Whereas, in the case of LSD and brom-LSD, *in vitro* tests on the uterus and the renal vessels of the cat give identical results, acetyl-LSD is twice as effective in inhibiting the action of serotonin on the uterus as it is on the renal vessels. *In vivo* the effects of acetyl-LSD on the peripheral vessels and the bronchial musculature are of practically equal intensity, and this finding also applies to the potentiation of the action of barbiturates in the mouse.

Of no less interest than its serotonin antagonism is the ability of acetyl-LSD to produce psychic changes. It is certainly unexpected that brom-LSD produces no effects of this kind, whereas acetyl-LSD appears to have an activity of an order similar to that of LSD. So far we have had little experience with acetyl-LSD, but the results have been decidedly positive.

I now come to a discussion of the following questions: (1) Which compounds exert psychic effects? (2) Is there a parallel between serotonin antagonism and the effects on the psyche?

The answer to the first question is relatively simple (see FIGURE 2). Of all the derivatives of lysergic acid so far investigated, only the monoethylamide of *d*-lysergic acid (LAE), the diethylamide of *d*-lysergic acid (LSD-25), and the diethylamide of *d*-1-acetyl-lysergic acid produce changes in the psyche. Although the psychic effects of the 3 derivatives are qualitatively similar, there are considerable quantitative differences between them. In the case of the monoethylamide of *d*-lysergic acid, the average human dose required to produce psychic changes is 500 μg., whereas LSD and, probably, also acetyl-LSD are effective in 8 to 10 times smaller doses. It is noteworthy, from the *chemical point of view*, that only the monoethylamide and the diethylamide, whether of lysergic acid or of acetyl-lysergic acid, are able to produce significant psychic changes. Thus there can be no doubt that the ethyl group is particularly important in this respect. In this connection, it may be mentioned that the monomethylamide and dimethylamide and the monopropylamide and dipropylamide do not possess any psychic action. On the other hand, both in animals and in man the 4 latter compounds are capable of eliciting autonomic actions in doses in which the monoethylamide, for example, is completely inactive. Thus, a dose of 50 μg. of the dimethylamide of *d*-lysergic acid is sufficient to elicit marked autonomic responses, whereas as much as 500 μg. of the monoethylamide of *d*-lysergic acid are necessary to produce similar effects. With the latter compound, however, simultaneous psychic changes occur, whereas

TABLE 2

COMPARISON BETWEEN THE PHARMACOLOGICAL ACTIONS OF LSD AND BROM-LSD

	LSD	Brom-LSD
Rabbit uterus and vagina *in vivo*	Contraction Approximately 1.5 times weaker than ergonovine (ergometrine)	No contraction In higher doses, inhibition of the spontaneous rhythm
Adrenolytic effect (seminal vesicle of guinea pig)	Approximately 50 times weaker than ergotamine	Approximately 5 times weaker than ergotamine
Blood pressure in the cat	Decrease	Very weak action, non-specific
Heart rate	Bradycardia	No effect
Eye, pupil	Mydriasis	No effect
Body temperature:		
Rabbit, dogs, cat	Rise (in all doses)	Decrease in high doses
Rat	Decrease; toxic doses: rise	Decrease (in all doses)
Heat production (calorimeter)	Primarily no increase; secondary rise	Not investigated
Blood sugar	Increased	No change
Behavior of normal mice	Excitation	Sedation
Amphetamine-excitation in the mouse	Potentiated	Inhibited
Effect on waltzing mice	Inhibition of waltzing due to excitation	Inhibition of waltzing due to to sedation
Potentiation of pentothal effect in the mouse	Marked	Present but weaker than with LSD
EEG in the rabbit	Activation	No activation
Chromatophores (Poecilia)	Spreading	2.5 times as strong as LSD
Psychic action in man	Very pronounced	Absent
Toxicity L.D.$_{50}$ Mouse *i.v.*	L.D.$_{50}$ 46 mg./kg.	20 mg./kg.
Rat *i.v.*	16.5 mg./kg.	—
Rabbit *i.v.*	0.3 mg./kg.	6 mg./kg.

the monomethylamide and dimethylamide in doses sufficient to elicit autonomic responses cause no changes in the psyche.

The second question, whether there is either an interference or a causal relationship between the inhibition of serotonin and the psychic effect, is more complicated than would appear from the investigations that have been described. Perhaps this is because hypotheses have been formulated regarding this relationship that appear to be plausible but which, in our opinion, are far from being proved.[13, 15, 16] All the substances that have been investigated, ncluding LSD and its derivatives, possess, to a certain extent, the property of nhibiting serotonin, particularly *in vitro*. From the studies of Woolley,[17] and others, we know that many serotonin antagonists are effective *in vitro*, but not *in vivo*. Consequently, the first condition that a serotonin antagonist should fulfill is that it should also inhibit serotonin *in vivo*. Both LSD and brom-LSD and, as far as we can tell at present, also acetyl-LSD exhibit this property to a significant degree. For example, as we have already indicated, they are able to inhibit the potentiating effect of serotonin on barbiturates. Of all the derivatives of *d*-lysergic acid so far investigated, however, only LSD, acetyl-LSD, and the monoethylamide of lysergic acid (LAE) exert a definite effect on the psyche. Although brom-LSD inhibits the potentiating effect of serotonin on barbiturates in the same manner as LSD, it is without any effect upon the psyche. In experiments carried out on 19 normal subjects, all of whom worked in our laboratories, doses of up to 650 μg. were administered without causing any symptoms other than those referable to the autonomic nervous system. Hirsch, Jarvik, and Abramson[18] have also found that after the administration of doses of brom-LSD of the order of 5 to 7 μg./kg. no significant changes occurred whereas, with LSD, psychic effects were observed after the administration of only 1 μg./kg. Still more striking are the results of a therapeutic trial of brom-LSD in carcinoid patients. Waldenström[19] administered doses of up to 1.5 mg. (1500 μg.) without causing side effects of psychic changes, but also without producing any significant decrease in the symptoms of the disease. In one patient, Snow[20] administered up to 7.5 mg. of brom-LSD daily over a period of 3 weeks without observing any changes in the psyche. The therapeutic effect on the symptoms of the disease was insignificant. The dose of 7.5 mg. or 7500 μg. is, however, 100 times the dose of LSD needed to produce significant changes in the psyche. We therefore arrive at the following conclusions: that while brom-LSD has a highly specific anti-5-hydroxytryptamine effect in doses that fully antagonize the action of 5-hydroxytryptamine, brom-LSD shows no signs of antihistaminic, adrenergic, or cholinergic blocking effect.

As the hypothesis of the cerebral functions of 5-hydroxytryptamine is based to a great extent on the fact that lysergic acid diethylamide is a strong antagonist of 5-hydroxytryptamine, the present finding that brom-LSD is as active as lysergic acid diethylamide in antagonizing 5-hydroxytryptamine, but that it produces none of the mental disturbances, makes it necessary to reconsider this hypothesis. It cannot be argued that brom-LSD lacks cerebral actions because it does not penetrate into the brain tissue since the sedative action it produces is a central effect. In addition, after injection, brom-LSD could be detected in the same indirect way as lysergic acid diethylamide in extracts of the brain when tested for anti-5-hydroxytryptamine activity. Brain extracts of mice previously injected with either brom-LSD or lysergic acid diethylamide exerted an anti-5-hydroxytryptamine activity.

Our results with brom-LSD thus make it difficult to correlate the psychic effects of lysergic acid diethylamide with its anti-5-hydroxytryptamine property. At present, we are not justified in assuming a causal relationship between these 2 properties of lysergic acid diethylamide, although it may be found eventually that the 5-hydroxytryptamine in the brain is involved in the central actions of lysergic acid diethylamide. The mere existence of a pharmacological antagonism between lysergic acid diethylamide and 5-hydroxytryptamine, however, no longer provides evidence for the hypothesis that inhibition of the latter in the brain is the cause of the mental disturbances.

There is one final point I should like to mention. All of these compounds that produce changes in the psyche—LSD, LAE, and acetyl-LSD—also cause central autonomic effects. A thorough study of these effects in rabbits has shown that LSD elicits these responses in doses as small as those that cause psychic changes in man. The autonomic effects include rise in body temperature with a threshold dose of 0.5 μg./kg., hyperglycemia, mydriasis, piloerection, tachycardia, leucocytosis, and changes in the EEG that are characterized by disappearance of the spindle bursts and absence of slow wave activity. All of these effects are of a central nature, due to the stimulation of mesoencephalic and diencephalic structures, and they all have a pronounced *Sympathicotonic character*. They are suppressed both by ganglion-blocking agents (hexamethonium bromide) and by the hydrogenated alkaloids of the ergotoxine group (Hydergine). We emphasize this syndrome of sympathetic excitation produced by LSD for 2 reasons: first, brom-LSD does not cause this central stimulation of the sympathetic system and, second, this central sympathetic excitation produced by LSD may be considered the counterpart of the syndrome of central sympathetic depression produced by reserpine, which is characterized by the predominance of vagal functions and such symptoms as a fall in body temperature, bradycardia, miosis, and sedation. The actions of LSD and reserpine are of an opposite nature, not only with regard to autonomic functions, but also with regard to the psyche. Whereas the LSD psychosis is characterized by a hyperreactive condition and a schizophrenic-ike behavior, reserpine exerts a sedative effect and not infrequently causes depression. Whether LSD and reserpine act on the same central mechanism, but in different ways, and whether their effects are perhaps correlated with one another, can be decided only by further study. In this connection, attenion may be directed to the metabolism of 5-hydroxytryptamine (serotonin). According to Shore, Silver, and Brodie,[21] reserpine leads to a decrease in the ontent of serotonin in the brain. How LSD affects the cerebral metabolism f serotonin is not known but, as I have already mentioned, LSD is able to inibit the potentiating effect of reserpine on barbiturates. It is not yet clear whether serotonin plays any part in this mechanism. In any case, it is imporant that brom-LSD, in contrast to LSD, is not able to inhibit the potentiating ffect of reserpine on barbiturates. Although these findings may have taken s a stage further, the mechanism of the action of LSD on the psyche, and also he question of a possible connection between this action and serotonin metablism, still need much more investigation.

In conclusion we should like to say that in the animal, a central sympathicoimetic syndrome predominates and that psychic changes are probably present, ut difficult to interpret. In man, the psychic changes predominate, while ne autonomic symptoms are apparently subordinated. We assume, howver, that these 2 actions are not independent of each other, but are coupled n such a manner that their manifestation is effected by means of a common oordinative center. It would be desirable, therefore, that future clinical inestigations should include an analysis of autonomic symptoms as thorough s that presently being devoted to the psychic changes.

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Discussion of the Paper

Richard Tislow (*Wyeth Institute of Medical Research, Philadelphia, Pa.*): Have you tried brom-LSD (the 2-brom-diethylamide of lysergic acid) clinically as an antagonist of LSD-25 to determine whether it prevents the hallucinogeni effects of the latter compound?

Ernst Rothlin: No, as far as I know, this has not been tried clinically. We have tried, however, to prevent LSD effects in the animal by pretreatment with brom-LSD (BOL 148), and we were able to show partial inhibition o the highly specific pyretogenic LSD effect by brom-LSD.

LYSERGIC ACID DIETHYLAMIDE ANTAGONISM OF CERTAIN DRUGS

By Barbara B. Brown
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The interactions of lysergic acid diethylamide (LSD) with various sub- ances have been the subject of considerable investigation. The interaction ost extensively explored has been that of LSD and serotonin because of the gh degree of specificity of this phenomenon. By a series of deductions from e competitive action of these 2 agents in isolated organs (Gaddum and Ha- eed[1] and Woolley and Shaw[2]) and from indirect evidence of their central ac- vities (Shore, Silver, and Brodie[3]), there has been a major effort to ascribe serotonin a key role in normal and abnormal cerebral functioning. Because marked species differences in reaction to serotonin and its hormonal charac- ristics, its true role in cerebral function has been difficult to define.

The present study, which deals with 2 methods for evaluating central LSD teractions, originated with the author's experience demonstrating the nor- alizing effect of intravenously administered azacyclonol (Frenquel) on LSD- duced psychoses (Brown, Braun, and Feldman[4]), and with the report that acyclonol occasionally relieves severe, acute psychoses in dramatic fashion abing[5]). Laboratory results at that time had indicated a potentiating effect LSD on the action of azacyclonol in prolonging hypnosis induced by hexo- rbital soluble (Brown, Braun, and Feldman[4]). These apparently contra- ctory results prompted a more detailed study of the central interactions nong LSD, serotonin, reserpine, and iproniazid (Marsilid), as well as aza- clonol.

Methods

Effect on hexobarbital sleeping time. Because of undue variation in both the ration of hypnosis induced by hexobarbital and the effects of drugs thereon und by this worker and by others, the following precautions were taken. oups of male Swiss albino mice obtained from a single source and each ani- al weighing between 18 and 22 gm., were used only on the second day through e fifth day after reaching the laboratory, since control studies showed that eping times were consistent on these days. Groups of 12 mice each were nployed for each level of dosage of each drug, and a control group was included ch time an experiment was conducted. Each set of experimental results s confirmed in some instances in additional experiments, but only one group experiments per dose level is reported here to show the variation expected any 1 experiment. The criterion for duration of sleeping time was the time m the loss of righting reflex to its re-establishment. The righting reflex s considered returned when the mouse righted itself within 30 seconds after ing placed on its back following occurrence of spontaneous righting. Stand- l errors of the means were calculated by the method of Miller and Tainter.[6]

Hexobarbital was always administered intraperitoneally in a dose of 100 g./kg. Serotonin, reserpine, and LSD were injected intraperitoneally, iproniazid was given subcutaneously, and azacyclonol was given orally. All agen were given 1 hour prior to the administration of hexobarbital. When LS was given in addition to the other compounds, the dose of LSD was alwa 10 mg./kg.

Effects on spontaneous activity of grouped mice. The method is an adaptatic of the method of Dews[7] in which groups of 5 mice each are placed in a sma box having 2 plastic sides through which a beam of light passes to a phot electric cell. A counter in the circuit totals the number of times the mice cro the beam. Counts are taken each 15 minutes to determine the time-actic course of the drugs, but total activity for a 90-minute period is used to sur marize effects for the various dose levels employed. The criterion for val results is the absence of abnormal locomotion during the 90-minute period.

All administrations of drugs were made as noted for the studies of hex barbital and, as before, when LSD was given in addition to the other con pounds, the dose of LSD was always 10 mg./kg. During the hour betwee drug administration and beginning of activity determinations the mice we kept in individual cages. Under these conditions, consistent results we obtained, and only 3 to 4 groups of mice were necessary per dose level of eac drug.

Results

Hexobarbital studies. A control study graphed in FIGURE 1 shows that, ove a wide dose range, LSD had no effect on duration of hypnosis induced by hexo barbital. The effects of serotonin and of reserpine are shown in FIGURE The intraperitoneal injection of serotonin (5 and 20 mg./kg.) 1 hour befor the administration of hexobarbital doubled the sleeping time, and doses 80 and 320 mg./kg. quadrupled the sleeping time. The intraperitoneal injec tion of LSD (10 mg./kg.), along with the various doses of serotonin, suppresse

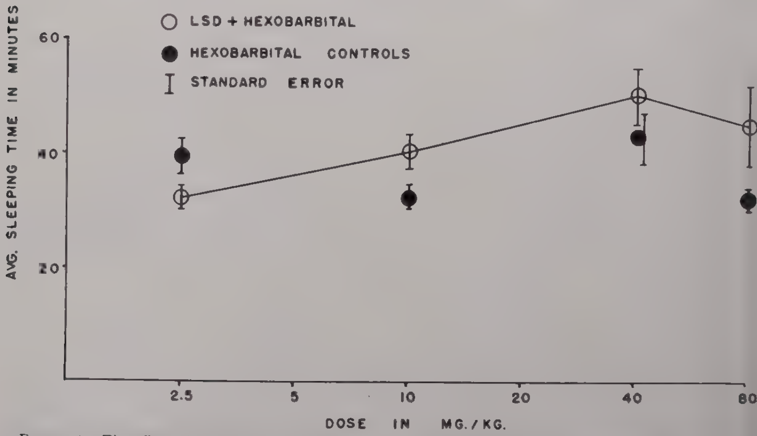

FIGURE 1. The effect of LSD on the duration of hypnosis induced in mice by hexobarbital. The LSD wa given intraperitoneally 1 hour before the administration of 100 mg./kg. of hexobarbital.

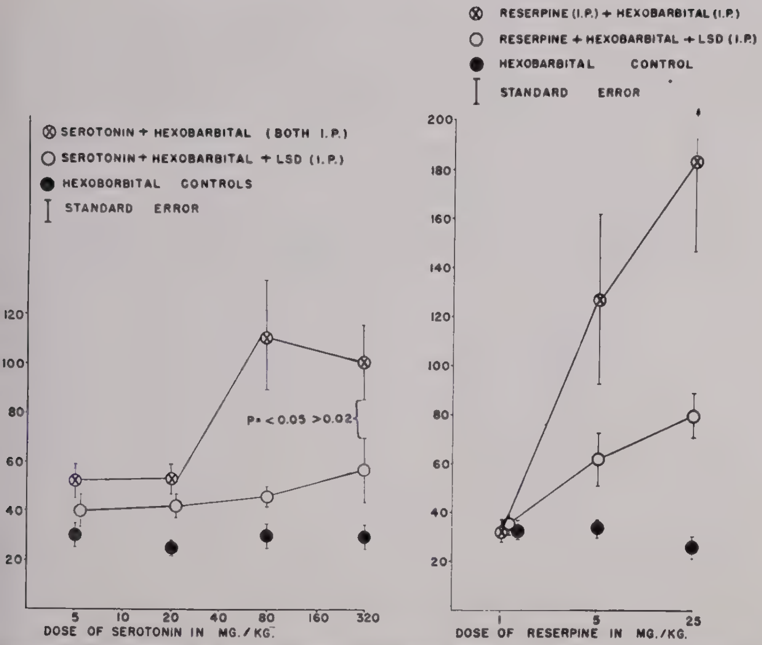

FIGURE 2. The changes in the duration of sleeping time induced by 100 mg./kg. of hexobarbital by serotonin, otonin plus LSD, reserpine, and reserpine plus LSD. The other drugs were given 1 hour prior to the adminis- tion of the hexobarbital.

e potentiating effect of serotonin, although never entirely, irrespective of e dosage of serotonin. Doses of LSD (80 mg./kg.) had no greater effect reducing the potentiation.

The potentiating action of reserpine on sleeping time induced by hexobar- tal, while considerably greater than that of serotonin, was antagonized to e same degree as that of serotonin by LSD.

The amine-oxidase inhibitor, iproniazid, produced a remarkable prolonga- on of hexobarbital hypnosis in doses of 50 mg./kg. and higher (FIGURE 3). SD exerted no effect upon this action of iproniazid, even where the prolonga- on of sleep was considerably less than maximal.

Azacyclonol (FIGURE 3), which is mildly depressant in mice, but stimulating dogs and cats, also caused a prolongation of hexobarbital hypnosis. Sleep- g times were approximately doubled, regardless of the dosage of azacyclonol. somewhat greater increase of sleeping time was obtained when azacyclonol as given 4 hours prior to the administration of hexobarbital. In the pres- ce of LSD, sleeping times for azacyclonol plus hexobarbital show a tendency increase, and the increase is of importance at the 260 mg./kg. dose level) per cent of the oral L.D.$_{50}$). The increase is of a similar order when the se of LSD is increased to 80 mg./kg.

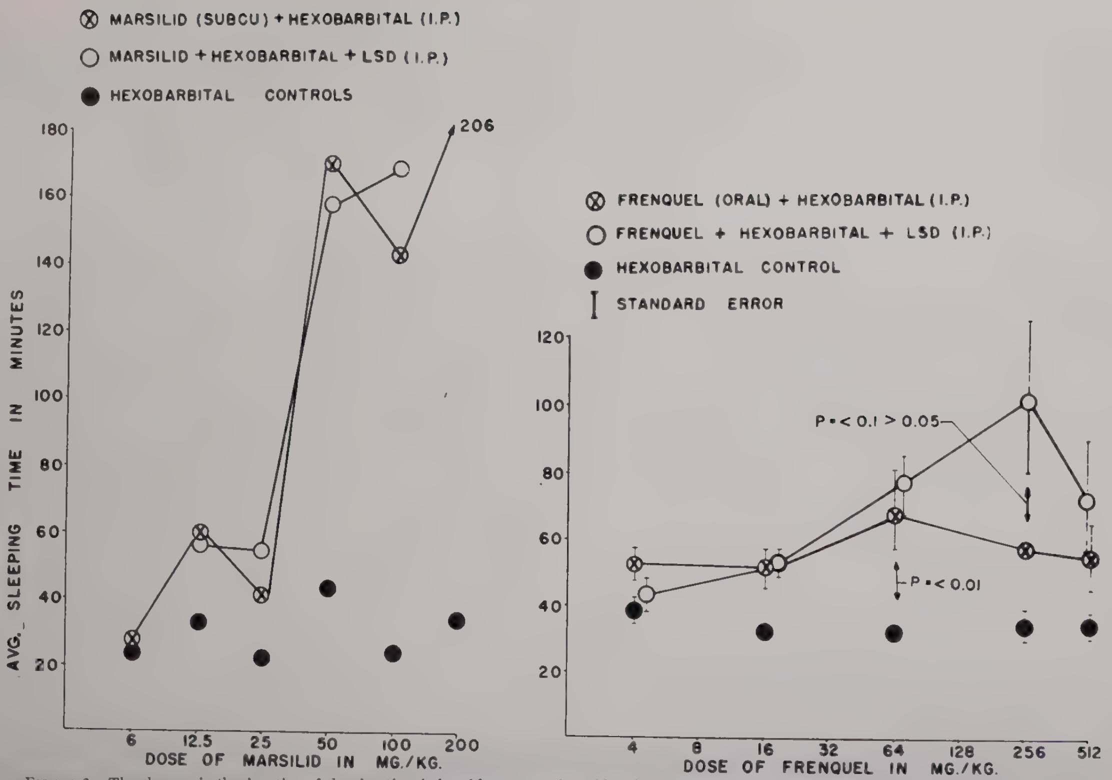

FIGURE 3. The changes in the duration of sleeping time induced by 100 mg./kg. of hexobarbital by iproniazid, iproniazid plus LSD, azacyclonol, and aza-

In additional experiments it was demonstrated that serotonin, iproniazid, nd azacyclonol all reinduce hypnosis when injected intravenously into mice ufficiently aroused from hexobarbital to show the escape response. All 3 of hese agents were effective in minimal doses, causing prolongation of sleep nduced by hexobarbital. Reserpine was not tried because of its known slow nset of action.

Studies of spontaneous activity. The control study for LSD is graphed in FIG-RE 4. The dose of LSD used in interaction experiments had no effect on the pontaneous activity of grouped mice 1 hour following its administration. The ntense, uncoordinated stimulation occurring shortly after the injection of LSD s of short duration, but the fact is important that no uncoordinated activity

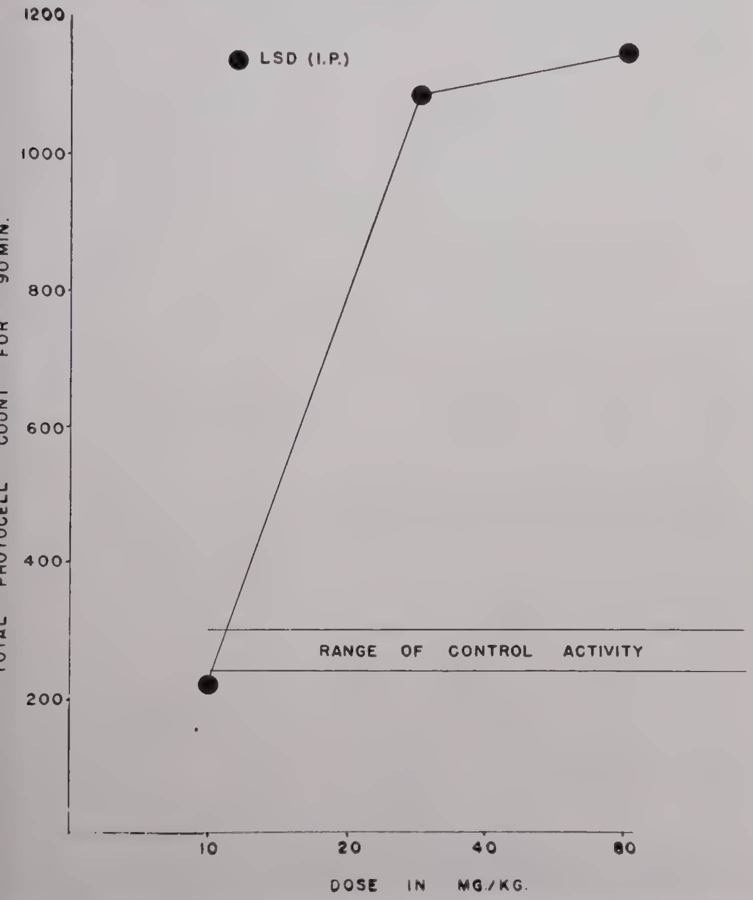

FIGURE 4. The effect of LSD given 1 hour prior to testing on the spontaneous activity of mice, as measured the photocell-counter technique.

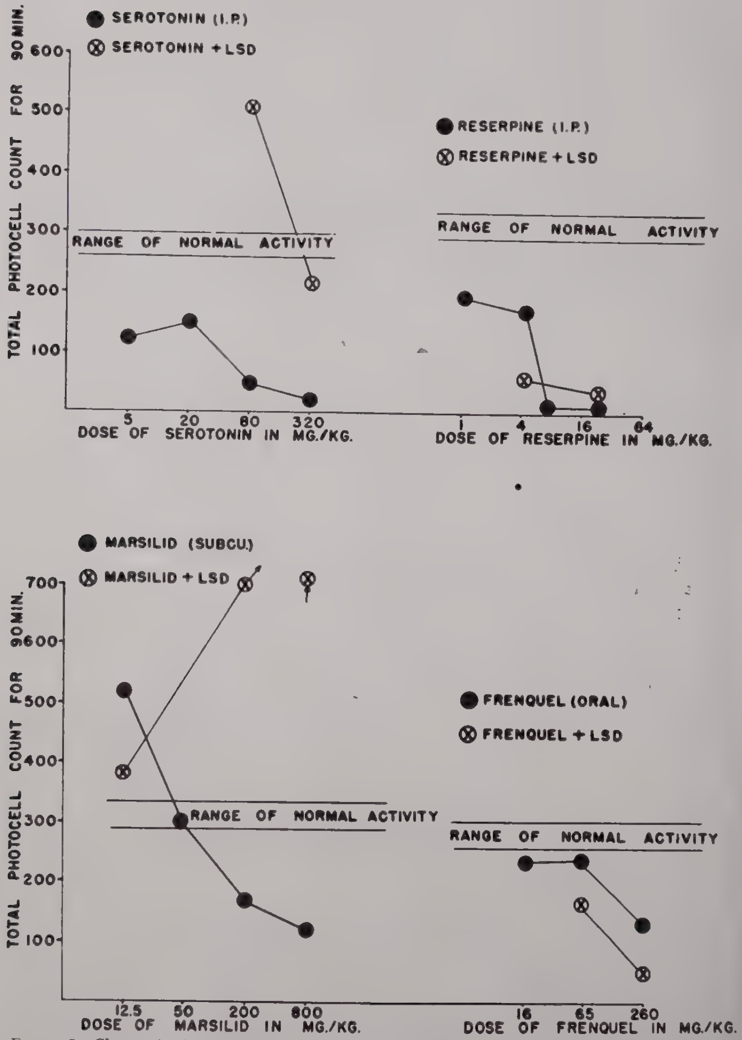

FIGURE 5. Changes in the spontaneous activity of mice, recorded by the photocell-counter technique, induced by serotonin, reserpine, iproniazid, and azacyclonol, and the effect of LSD on the changes of activity induced by these substances.

was noted 1 hour after measurements were started, even with the 80 mg./kg. dose. Doses of 28 and 80 mg./kg. produced a fourfold increase in spontaneous coordinated activity which persisted for at least 3 to 4 hours.

The effects of serotonin and of reserpine on the spontaneous activity of the grouped mice is shown graphically in FIGURE 5. Serotonin consistently depressed activity, and the dose-effect curve essentially resembles that for hexobarbital potentiation. In the presence of a nonstimulating dose of LSD (10 mg./kg.), the depression was converted into a stimulation at the 80 mg./kg. level of serotonin. An increased effectiveness of LSD is evident, even in the presence of an overwhelming dose of serotonin (320 mg./kg.). In contrast, equivalent degrees of depressed activity induced by reserpine were unaffected by the same dose of LSD. A dose of LSD of 80 mg./kg. was required to raise reserpine depression to levels of normal activity.

Iproniazid does not depress spontaneous activity until doses that also markedly prolong hexobarbital hyponosis are reached (FIGURE 5). As in the case of serotonin, the depression induced by iproniazid was converted into stimulation by a nonstimulating dose of LSD. The increase in the degree of stimulation as the dose of iproniazid is increased while LSD dosage remains constant is particularly interesting, since at the 800 mg./kg. dose plus LSD, the hyperactivity remained coordinated.

Azacyclonol (FIGURE 5) requires a large dose to induce measurable depression, although doses as small as 16 mg./kg. potentiate the sleeping time induced by hexobarbital. As in the studies of hexobarbital, the addition of LSD to azacyclonol increased the depressant action.

Discussion

The differences in LSD interactions with serotonin, reserpine, iproniazid, and azacyclonol seen with the 2 types of measurement of central depressant actions pose some interesting questions, particularly with respect to serotonin and the role postulated for this substance by a number of investigators. In the absence of hexobarbital, why is the depressant action of serotonin converted into a stimulant action by LSD? It would appear that serotonin renders the central nervous system more sensitive to LSD stimulation. The failure of LSD to affect reserpine similarly supports this conclusion indirectly. There is also the possibility that LSD displaces serotonin at the receptors, the displaced serotonin being either inactive or metabolized, and the consequent lack of effective serotonin results in stimulation. Either mechanism could apply also to results of the studies of hexobarbital with serotonin. The fact that a nonstimulating dose of LSD antagonizes the hexobarbital potentiating effect of any dose of serotonin to the same degree also suggests that it is the lack of effective serotonin at the receptors that results in stimulation.

LSD antagonizes the hexobarbital-potentiating action of reserpine, but not the depressant action of reserpine on spontaneous activity. If the entire central activity of reserpine were mediated through serotonin release, as proposed by Pletscher, Shore, and Brodie,[8] the depression induced by reserpine should also be converted into stimulation. Since it is not, another or an additional mechanism that does not involve serotonin is indicated.

The depression of spontaneous activity produced by iproniazid and the conversion of this activity to coordinated hyperactivity by LSD resembles, in some respects, that obtained for serotonin. Consequently it may be reasoned that these responses are a result of the accumulation of serotonin by the amine oxidase inhibitory action of iproniazid, and that the resulting serotonin either increases central sensitivity to LSD or is displaced by LSD at the receptors resulting in a loss of effective serotonin. It may be argued that endogenous serotonin accumulated by the action of iproniazid gives a clearer picture of the serotonin-LSD interaction than results obtained with administration of massive doses of serotonin. The hexobarbital-potentiating action of iproniazid, being much in excess of that produced by either serotonin or reserpine, along with the inability of LSD to affect the potentiation, indicates that this activity of iproniazid takes place through a mechanism unrelated to that involving serotonin.

Azacyclonol not only potentiates sleeping time induced by hexobarbital, but this activity is in turn potentiated by LSD. It thus appears that serotonin is not important in this depressant action of azacyclonol, and that azacyclonol, unlike serotonin, does not make the mouse sensitive to the effects of LSD. Indeed, this may be a real antagonism of LSD by azacyclonol which may either prevent the increase in central sensitivity to LSD caused by serotonin or prevent LSD from displacing serotonin at the receptors. It is also possible that LSD increases sensitivity of receptors to azacyclonol.

Conclusions

The interactions of LSD with serotonin, reserpine, iproniazid, and azacyclonol, as determined by measuring the degree of spontaneous activity induced in mice, do not correlate with the interactions as determined by measuring the effect on the duration of hypnosis induced by hexobarbital. The depression of spontaneous activity of mice by serotonin is converted into stimulation by a nonstimulating dose of LSD, whereas the same dose of LSD prevents the prolongation of hypnosis induced by hexobarbital to the same degree, irrespective of the dose-effect of serotonin. The potentiation of hexobarbital hypnosis by reserpine is similarly antagonized by LSD. The simple depression of mouse coordinated activity is not affected by LSD, however, and, indeed, the effects here are additive. The LSD-iproniazid interaction is similar in some respects to that for serotonin as evaluated by its effects on the spontaneous activity of mice, but the hexobarbital potentiating action of iproniazid is unaffected by LSD. In contrast, LSD increases the depressant characteristics of azacyclonol in both types of studies. The differences in the interactions found by the 2 methods of measurement suggest several different possible mechanisms for these substances, as noted in the discussion.

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THE ACTION OF TRANQUILIZERS ON BRAIN POTENTIALS AND SEROTONIN

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During the past 4 years a number of tranquilizing agents have been used successfully in the treatment of neuroses and psychoses. The most important of these are: chlorpromazine, a phenothiazine derivative prepared as one of a series of antihistaminic and adrenolytic agents; reserpine, an alkaloid originally introduced as a hypotensive agent; benactyzine, a benzilic acid ester with potent anticholinergic action; and meprobamate, a simple propanediol dicarbamate derivative that is devoid of autonomic effects. It is of interest that compounds so dissimilar in chemical structure and pharmacological action should all be effective as tranquilizers.

Brodie and his associates have shown recently that the administration of reserpine markedly decreases the serotonin (5-hydroxytryptamine) levels in the brain (Pletscher, Shore, and Brodie, 1956). They also observed that the excretion of 5-hydroxyindoleacetic acid (5HIAA), a metabolite of serotonin, was markedly increased following the administration of reserpine (Shore, Silver, and Brodie, 1955). These investigators postulated a causal connection between the effectiveness of reserpine as a tranquilizer and the liberation of bound serotonin from the brain. It was therefore of interest to see whether other tranquilizers would also affect serotonin metabolism.

Certain tranquilizing agents are known to antagonize serotonin-induced contractions of smooth muscle (Costa, 1956). We have compared the actions of these 4 tranquilizing drugs in their effect on contractions of smooth muscle induced by serotonin and acetylcholine. Chlorpromazine, reserpine, and benactyzine potently antagonized the contractions produced by acetylcholine and serotonin, while meprobamate was ineffective against both stimulants.

This report also includes a summary of our studies on the effects of the tranquilizers on the electrical activity of the brain. We have found, in the cat, that meprobamate in low doses synchronizes the electrical activity recorded from the thalamus without causing important changes in other areas of the brain (Hendley, Lynes, and Berger, 1954, 1955, 1956). In the present report we have compared meprobamate and other tranquilizers, especially chlorpromazine, in their effect on spontaneous electrical potentials recorded from the cortex and various subcortical structures.

Effect of Tranquilizers on Excretion of 5-Hydroxyindoleacetic Acid

Male rats weighing 200 to 300 gm. were used in this study. Urine samples from 10 animals were pooled in each experiment. Each animal was given 5 ml. of water per 100 gm. body weight to assure a sufficient excretion of urine. Drugs were administered intraperitoneally in the following doses: reserpine

5 mg./kg., meprobamate 200 mg./kg., chlorpromazine 5 mg./kg., and benactyzine 5 mg./kg. There were 17 control runs in which only water was given. Urine was collected continuously from each group for a period of 24 hours, and samples were analyzed for 5HIAA at 2, 4, 6, 8 and 24 hours according to the method of Udenfriend, Weissbach, and Titus (1956).

The results are summarized in FIGURE 1, where the total excretion of 5HIAA in micrograms per group of rats is plotted as a function of time. Reserpine gives values differing significantly from the controls at 4 hours and beyond. At 4 and 6 hours, the *P* value was 0.05, and at 8 and 24 hours *P* was less than 0.01. Meprobamate, on the other hand, did not show significant differences from control values at any time. Benactyzine and chlorpromazine, like meprobamate, had little or no effect on 5HIAA excretion. In FIGURE 2, we have plotted the ratio of the amount of 5HIAA excreted to the volume of urine at various times after the administration of the drugs. The marked increase in excretion of 5HIAA after the administration of reserpine is again apparent. It is also shown that the other drugs did not affect significantly the 5HIAA excretion, even when the excretion is considered in relation to the volume of urine.

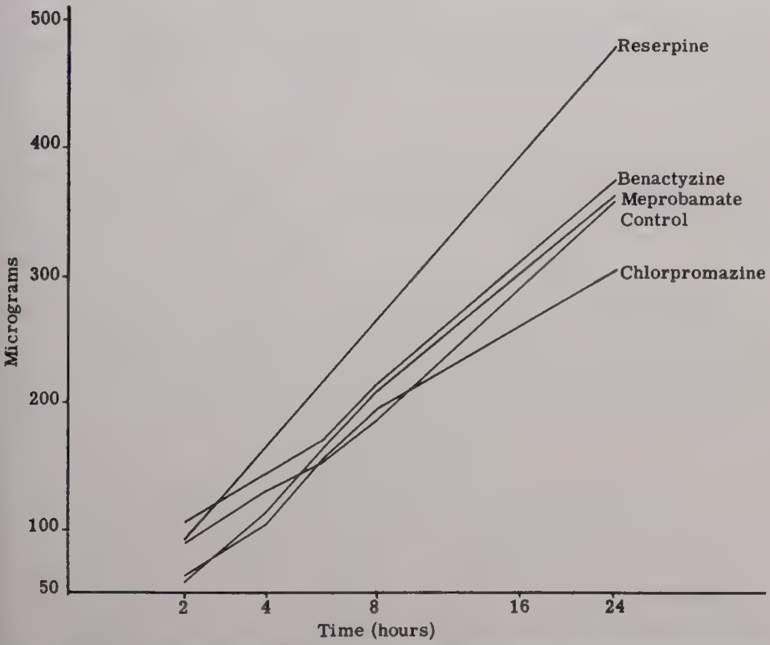

FIGURE 1. The total excretion of 5-hydroxyindoleacetic acid in micrograms per group of rats as a function of time. The curve for reserpine differs significantly from the controls at 4 hours and beyond. (P is less than 0.05.) No other drugs showed significant differences.

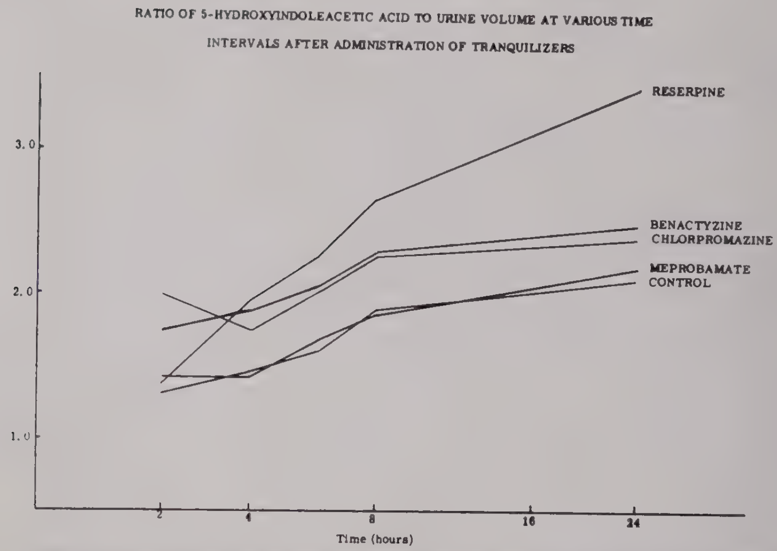

FIGURE 2. A plot of the ratio of the total micrograms of 5-hydroxyindoleacetic acid to the volume of urine at various times after the administration of tranquilizers. Reserpine groups deviate significantly from controls at all times after 4 hours. (P is less than 0.01.) None of the other drugs shows significant differences.

Effect of Tranquilizers on the Isolated Rat Colon

Segments of the colons of male rats were suspended in a conventional test bath in Ringer's solution at 37 or 38°C. Submaximal contractions of the muscle were produced by acetylcholine chloride (0.05 γ/ml.) or serotonin creatinine sulfate (0.05 to 0.10 γ/ml.). After several contractions were obtained, drugs were added and allowed to remain in the bath for 3 minutes before test doses of acetylcholine and serotonin were added. The results are summarized in TABLE 1. Chlorpromazine was the most potent agent in blocking serotonin contractions, and it was also quite effective against acetylcholine. Reserpine was somewhat less potent than chlorpromazine against both challenging drugs. Meprobamate was a very weak antagonist, being about 100 times less effective than chlorpromazine. Benactyzine was by far the most potent in blocking

TABLE 1

THE EFFECT OF TRANQUILIZERS ON ACETYLCHOLINE AND SEROTONIN STIMULATION OF THE ISOLATED RAT COLON

	Acetylcholine*	Serotonin*
Chlorpromazine	1	0.5
Reserpine	4	4
Meprobamate	100	100
Benactyzine	0.05	1

* Dose in γ per ml. that completely blocks response to acetylcholine or serotonin stimulation.

acetylcholine effects, and this compound was nearly as effective as chlorpromazine against serotonin. These results indicate that there is little correlation between the tranquilizing effectiveness of these agents and their ability to block the effects of serotonin on smooth muscle.

Effect of Tranquilizers on the Electrical Activity of the Brain

Recordings of electrical activity of the brain were made in cats prepared for recording under ether anesthesia, immobilized with *d*-tubocurarine, and maintained on local anesthesia and artificial respiration for from 5 to 8 hours. The dose of *d*-tubocurarine did not exceed 0.5 mg./kg. per hour. Subcortical recordings were made from bipolar concentric electrodes, with 1 mm. separation in the vertical dimension. Five of these electrodes usually were placed in each animal. The cortical electrodes were solder discs of about 2 mm. diameter, usually placed epidurally. A Grass model 3D encephalograph was used for recording. The paper speed was usually 6 cm./sec. in order to show the fast activity characteristic of this species. All drugs were administered by the intravenous route. A total of 48 adult cats were used in these studies. Thirty of them received meprobamate, 8 chlorpromazine, 4 reserpine, 6 amobarbital, 2 pentobarbital, and 2 benactyzine. At the end of each experiment, the brain was fixed and sectioned to determine the exact electrode locations.

In the recording of electrical activity from cortical and subcortical structures, meprobamate produced the most characteristic changes. These changes consisted of synchronization of electrical activity recorded from the thalamus. This is shown in FIGURE 3. The recording from the nucleus ventralis lateralis of the thalamus showed a low-voltage, featureless record. After the intravenous injection of meprobamate (20 mg./kg.), 10 to 15 cycles/sec. waves are seen clearly. These waves typically show waxing and waning and, in some cats, they appear as isolated spindles. Changes in the thalamic record usually are seen within 5 minutes after the injection of meprobamate, reach a peak within 20 minutes, and begin to subside within an hour. In contrast to those from the thalamus, records from the hypothalamus and the caudate nucleus show no change at this level of dosage, and those from the cortex show only a very slight slowing. All areas show a slowing and an increase in amplitude after a dose of 120 mg./kg. The cortical recordings are bipolar, from the left sensorimotor area to the left occipital area. Recordings also were made in each experiment from the auditory area. Unipolar recordings with the indifferent electrode on the frontal bone were also taken. The effects of drugs seemed to be similar in all cortical loci, so that only single representative cortical tracings are shown. In other experiments we found changes with meprobamate comparable to those shown in FIGURE 3 in other thalamic nuclei, including the nucleus centrum medianum, the centralis medialis, the ventralis posteromedialis, and even in the lateral geniculate nucleus.

In contrast, neither the anterior nor the mid-hypothalamic loci showed specific changes with the administration of meprobamate, nor did the anterior mesencephalic reticular area. A number of rhinencephalic structures were entered, including the septum, fornix, basal olfactory area near the diagonal

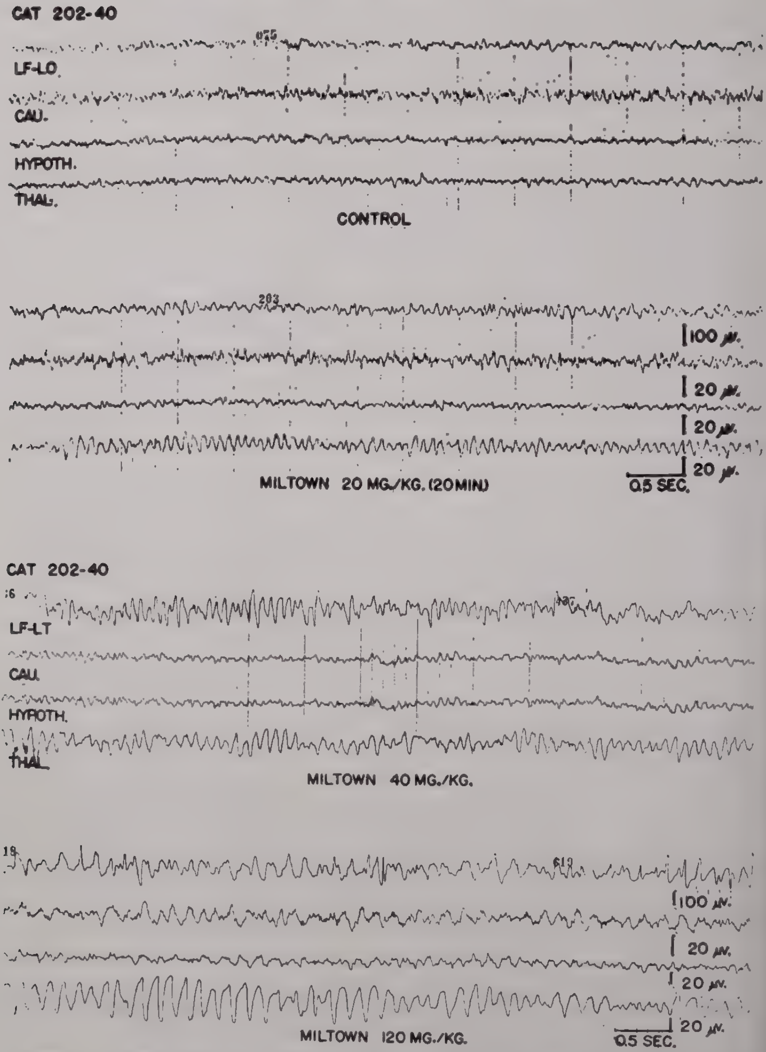

FIGURE 3. The effect of meprobamate (Miltown) on the electrical activity recorded between the left frontal (LF) and left occipital (LO) cortex, caudate nucleus (CAU), hypothalamus (HYPOTH), and nucleus ventralis lateralis of the thalamus (THAL). A dose of 20 mg./kg. shows synchronization of thalamic activity. At higher doses all areas are affected.

band of Broca, amygdaloid nuclei, and hippocampus. None of these areas showed changes in spontaneous electrical activity with low doses of meprobamate, and injury discharges from the fornix and amygdala were unaffected by this agent.

Chlorpromazine at a dose of from 1 to 15 mg./kg. produced irregular slowing in the cerebral cortex, with outbursts of 7 to 10 cycles/sec. of activity and occasional slow waves of 2 or 3 cycles/sec. This slowing was usually accompanied by similar changes in the thalamic recordings, although the outbursts of slow activity were not always synchronous with those in the cortex. This is shown in FIGURE 4, where outbursts of slow activity are seen in the thalamic and cortical recordings after the administration of chlorpromazine, while recordings from the tuberculum olfactorium and the amygdaloid nuclei show no apparent change.

Changes such as this were found in 6 of the 8 animal preparations used. The magnitude of the effect did not seem to be correlated with the dose. One cat showed no changes seen after 3 successive doses of 5 mg./kg. each. Another cat showed a considerable amount of slow activity and spindling before the drug was given, and possible drug effects were obscured. In 1 experiment, a recording of blood pressure and the electrocardiogram gave no evidence that cardiovascular changes might be the cause of the chlorpromazine effect. In 4 of the cats, recordings were taken from the anterior and mid-hypothalamus. Slow activity was seen in 2 of these preparations, but no specific effects were noted.

With reserpine, at the rather high dose of 2.0 to 2.5 mg./kg., there was evidence of slowing and spindling in the cortical and thalamic records in 3 of 4 animals. The effects were not as pronounced as those obtained with chlorpromazine or meprobamate, however.

Benactyzine has been administered in 2 cats. At a dose of 0.5 mg./kg. this compound produced marked effects on spontaneous electrical activity of the brain. These are illustrated in FIGURE 5 where the fast activity of the cortex is greatly reduced after the administration of benactyzine, while irregular out-

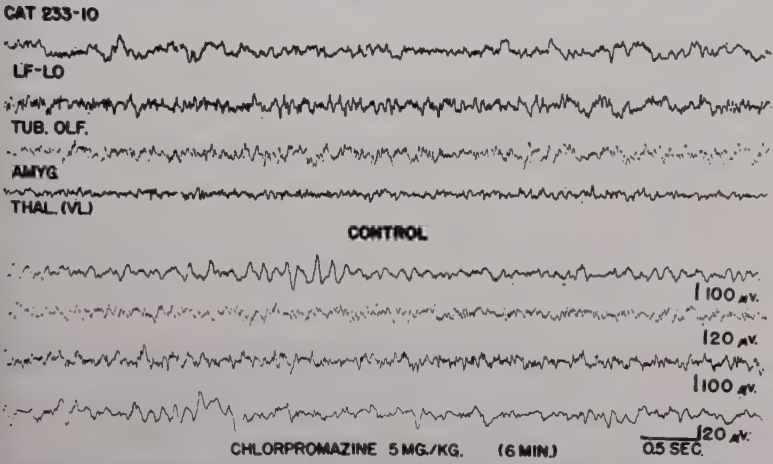

FIGURE 4. The effect of chlorpromazine on electrical activity. Note the appearance of slow activity in the cortex (LF-LO) and thalamus, nucleus ventralis lateralis (VL). The tuberculum olfactorium (TUB OLF) and amygdaloid nuclei (AMYG) show no changes.

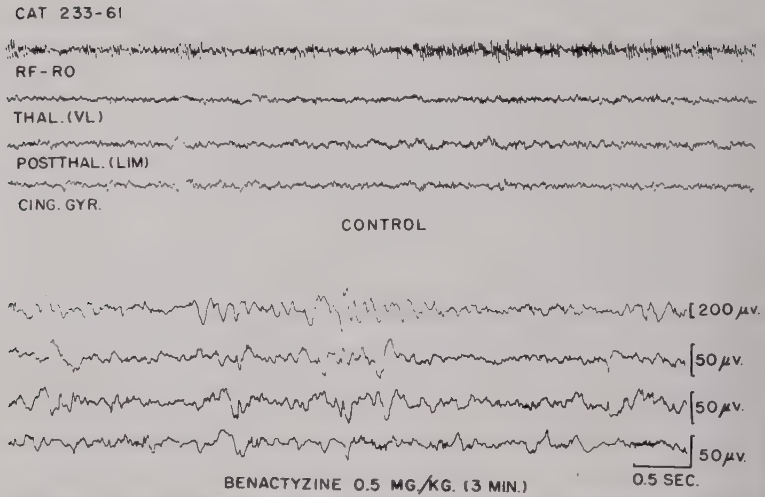

FIGURE 5. The effect of benactyzine on cortical and subcortical recordings. Irregular slow activity appears most prominent in the surface recording from the cortex (RF-RO), but it is also apparent in the posterior cingulate gyrus (CING GYR) and the thalamus, including the posterior border of the thalamus in the nucleus limitans (LIM).

bursts of 8 to 15 cycles/sec. are seen in the cortex and in the subcortical areas as well.

The recordings of spontaneous electrical activity from cortical and subcortical areas show that all the tranquilizing agents produce effects that are distinct from those of the barbiturates. Meprobamate seems to have a somewhat selective action on the thalamus. This compound produced an increase in amplitude and slowing in a number of ventral and posterior thalamic areas. We have not been able to localize the site of action of the drug in any definite group of nuclei. Direct sensory-projection systems cannot be depressed greatly by meprobamate, since no sensory impairment is apparent in animals or reported by humans at normal dosage, and since the auditory responses in the cortex of our cat preparation were affected only at very high doses.

In contrast to the thalamus, a number of other subcortical areas failed to show specific effects of low doses of meprobamate, including the hypothalamus and several parts of the rhinencephalic or "limbic" system that project to the hypothalamus. Evidence has been accumulating that the limbic system may be vitally important in emotional processes (MacLean, 1949; Gastaut, 1952; Klüver, 1952). Perhaps meprobamate is affecting this system in ways that are not evident in our gross recording of spontaneous activity. It may be significant, however, that this compound, in contrast with other tranquilizing agents such as chlorpromazine, reserpine, and benactyzine, has no direct effect on the autonomic nervous system, yet it seems specifically to be effective clinically in relieving the emotional disorders of anxiety and tension.

Chlorpromazine produced more generalized effects on electrical activity than did meprobamate. Our results are similar to those of Monroe *et al.* (1955) in cortical and subcortical recordings from humans and monkeys. Comparable cortical effects have been found in the monkey (Das, Dasgupta, and Werner, 1954) and in the cat (DeMaar and Martin, 1956).

Reserpine seems to have somewhat less effect on electrical activity than does chlorpromazine. Schneider and Earl (1954) have found that this agent produced minimal effects on the EEG of monkeys, although Monroe and his associates found it comparable to chlorpromazine in their studies. Preliminary indications are that benactyzine causes a generalized increase in slow activity and blockage of EEG arousal to sensory stimulation. These effects are somewhat like those of atropine (Funderburk and Case, 1951; Wikler, 1952; Rinaldi and Himwich, 1955).

Summary and Conclusions

Chlorpromazine, meprobamate, and benactyzine did not cause an increased excretion of 5-hydroxyindoleacetic acid in rats. The previously reported results (Shore, Silver, and Brodie, 1955) that reserpine markedly increased the excretion of 5-hydroxyindoleacetic acid in other species have been confirmed in rats.

Chlorpromazine, reserpine, and benactyzine powerfully antagonize contractions produced by serotonin or acetylcholine in the isolated rat colon. Benactyzine is the most potent acetylcholine antagonist, while chlorpromazine and reserpine show little specificity. Meprobamate antagonizes the stimulants only in large doses.

In the waking cat, meprobamate at a dose of 20 mg./kg., given intravenously, caused synchronization of the spontaneous electrical activity of the thalamus. Ten to 20 cycles/sec. waves appeared in long runs or in short spindles. The cerebral cortex was relatively unaffected, and no changes were seen in the hypothalamus or in various parts of the rhinencephalic or limbic system. At higher doses of meprobamate, all cortical and subcortical recordings showed slowing and spindling. Chlorpromazine, at doses of 1 to 15 mg./kg., caused slowing in both cortical and thalamic recordings. The effect did not seem to be correlated with the dose. Reserpine produced somewhat similar but less definite effects. Benactyzine, 0.5 mg./kg., produced generalized irregular slowing in the brain and blocked EEG arousal from sensory stimulation.

The results indicate that various tranquilizers that have been found to be effective clinically may produce varying effects on the metabolism of serotonin and on brain potentials. Correlations between these pharmacological properties and clinical effectiveness are not apparent at the present time.

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THE ANALEPTIC ACTION OF LYSERGIC ACID DIETHYLAMIDE ON RESERPINE-SEDATED MICE*

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Our laboratory is interested in the toxicity of reserpine, and we have studied many agents in an effort to decrease the mortality rate of mice given near-lethal doses of reserpine. The following observations made during this study may be of general interest to the readers of this monograph.

Reserpine produces a marked depression or stupor in mice, a condition characterized by a physical state resembling sleep, with failure to respond to stimuli such as noise, prodding, etc.[1, 2] The mice retain their righting reflex, however, thus distinguishing depression from hypnosis. As indicated by the data presented in TABLE 1, when the reserpine-sedated mice are given *d*-LSD or *d*-amphetamine a rapid response to the analeptic agents is observed. The reserpinized mice given an analeptic show a burst of spontaneous activity and an increased awareness and response to stimuli. The duration of the analeptic action of these agents is related to the dose administered. Both *d*-LSD and *d*-amphetamine produce equal responses on an equimolar concentration basis. *d*-Deoxyephedrine is as effective an analeptic as is *d*-LSD and *d*-amphetamine, but *l*-amphetamine and the monoethylamide of lysergic acid (LAE) are much less active as analeptics. Other compounds studied, such as *d*-2-brom-LSD (BOL-148), *l*-LSD, mescaline, Marsilid, serotonin, caffeine, metrazol, picrotoxin, strychnine, atropine, acetylcholine, epinephrine, levarterenol, dibenamine, and Ritalin (phenyl-[α-piperidyl] acetic acid methyl ester) fail to demonstrate analeptic activity even though the doses used frequently range high enough to include convulsant levels of the compounds.

Cerletti and Rothlin[3, 4] report that both *d*-LSD, which produces psychic disturbances, and *d*-2-brom-LSD, which does not produce abnormal mental reactions, are strong inhibitors of serotonin *in vitro* and *in vivo*. *d*-LSD[3, 4, 5] and *d*-2-brom-LSD[3, 4] have been detected in brain tissue, but only *d*-LSD was found to be active as an analeptic on reserpine-sedated mice. These data do not appear to be consistent with the hypothesis that the sedation produced by reserpine is mediated through serotonin.[6, 7, 8]

It has been shown by Rothlin[4] and by Rothlin and Cerletti[3] and confirmed in our laboratory that both *d*-LSD and *d*-2-brom-LSD are effective agents for inhibiting the prolongation of barbiturate anesthesia by serotonin. The data presented in TABLE 2 show that both *d*-LSD and *d*-amphetamine are potent agents for inhibiting the prolongation of pentobarbital anesthesia by reserpine. However, *d*-2-brom-LSD does not inhibit the anesthesia-prolonging action of reserpine. Similar results have been reported by Rothlin[4]. The experi-

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[*] The material presented in this discussion was obtained in collaboration with A. Goldin of the Laboratory of Chemical Pharmacology, National Cancer Institute, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

TABLE 1

Analeptic Action of LSD on Reserpine-Sedated Mice

Compound injected	Duration of analeptic action
1 μmol/kg.	*Minutes ± S.E.*
d-LSD	40 ± 1
l-LSD	0
d-2-Brom-LSD (BOL-148)	0
d-Amphetamine	44 ± 2

C57 black male mice (10 weeks old, 20 to 25 gm. body weight) were given reserpine (25 mg./kg.) by subcutaneous injection and allowed to become deeply depressed. Aqueous solutions of the compounds used were given intraperitoneally after 3 hours following the administration of reserpine. The animals were observed for spontaneous activity continuously throughout the experiment. The length of time of this spontaneous activity is defined as the duration of analeptic action. The analeptic agents were given at various dosages, the results were plotted as a dose-response curve, and the duration of analeptic action for 1 μmol of the compound estimated from the curve. The standard error reported is the average standard error for all dose levels of the compound. Five mice were used at each dose level, and the doses used were varied from 625 μg./kg. to 10 mg./kg. Control animals were prepared by administering reserpine and then giving no subsequent injections of possible analeptics and by giving the possible analeptics to animals that had not received reserpine.

TABLE 2

Reversal of Reserpine-Prolonged Pentobarbital Anesthesia

Additions	Duration of anesthesia	
	Pentobarbital	Reserpine and pentobarbital
	minutes ± S.E.	*minutes ± S.E.*
Control (no additions)	29 ± 6 (10)	72 ± 7 (10)
d-LSD		41 ± 6 (5)
d-2-Brom-LSD (BOL-148)		81 ± 10 (5)
d-Amphetamine		39 ± 6 (5)

C57 black male mice (8 to 10 weeks old, 15 to 25 gm.) were given reserpine (5 mg./kg.) subcutaneously 3 hours before inducing anesthesia with pentobarbital (60 mg./kg.; intraperitoneal injection). After 30 minutes of anesthesia, the mice were given the various compounds listed (5 mg./kg.). The duration of anesthesia is the time from the loss of the righting reflex until this reflex is regained. The number in parenthesis indicates the number of animals used in each group.

ments reported in this discussion suggest that reserpine sedation is not primarily due to a change in the effective concentration of serotonin.

Acknowledgments

We are indebted to J. Axelrod of the Laboratory of Clinical Sciences, National Institute of Mental Health, Bethesda, Md., for the gift of *d*- and *l*-amphetamine and *d*-deoxyephedrine, the CIBA Pharmaceutical Products, Inc., Summit, N. J., for the reserpine (Serpasil) and Ritalin, and to Sandoz Pharmaceuticals, Hanover, N. J., for the lysergic acid derivatives.

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Part IV. Psychotherapeutic Drugs: Clinical, Behavioral, and Pharmacological Effects

PHARMACOTHERAPY IN PSYCHIATRY: A REVIEW*

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Introduction

Although chlorpromazine and reserpine have been depicted as highly effective therapeutic agents by a legion of investigators, considerable disagreement still exists as to the actual value of these drugs. They have been considered by some as worthless, or as having only a sedative action, and as having no effect beyond the period of administration. In other quarters they have been regarded as superior to electroconvulsive and insulin-coma therapy and even as "curative" in nature. They have been described as both a valuable adjunct and as a hindrance to psychotherapy. It is the purpose of this report to review prevailing thought regarding these and other ataraxics and to present personal experience that supports both the sedative and the clearly greater-than-sedative function of one of these drugs—chlorpromazine.

Survey of Current Opinion

Scope. In the preparation of this report it was felt that in such a fast-moving and rapidly changing field much of the material that should be reviewed had not yet reached print or, if published, was in need of revision. Because of this it was felt necessary to communicate with numerous leading investigators in order to obtain all available current information. Accordingly, a letter inquiring into those aspects of this field considered to be the most controversial was sent to 80 investigators. The response to this effort was gratifying indeed. Ford[1] sounded out his staff and provided 7 pages of data. Overholser[2] polled his staff and prepared a 5-page report. Ayd[3] took enough time away from his busy practice to send a 4-page letter, several reprints, and unpublished manuscripts. Denber[4] made a critical analysis of his data and sent 5 pages of comments. Noce[5] put his work aside to transmit 8 pages. Tuteur[6] contributed 5 informative pages, and Forrer[7] and Gatski[8] each sent 4 pages. In all, 57 replies were received, and 45 of these contained pertinent data. These comments covered 126 typewritten pages and were accompanied by 40 reprints and 19 unpublished manuscripts. I am humbly appreciative of the efforts of my colleagues and regret that time and space do not permit the inclusion or acknowledgment of all the contributions.

Value of chlorpromazine and reserpine: unfavorable reports. In surveying the information thus obtained one is struck by the fact that in spite of the massive array of favorable and enthusiastic support of these drugs many converse opinions are held. Hohman[9] reports chlorpromazine to be "useless . . . with the exception of one or two dramatic cases." Jones[10] disagrees with the idea that these drugs may offer a substitute for electroconvulsive therapy and feels that they should be used only after the latter has failed. Tietz[11] feels that these drugs have no lasting effect and prefers to use electronarcosis with psychotherapy in the treatment of psychotics. Shanklin[12] regards these drugs as a temporary expedient that does not amount to treatment in itself. In his experience, paranoids still require electroconvulsive therapy and insulin in order to clear delusional patterns, regardless of the amount of drug given. Dickel,[13] in referring to the office practice of psychiatry, concludes that neither reserpine nor chlorpromazine aid in facilitating any psychiatric procedure and that in about 80 per cent of the cases either one may actually impede improvement and recovery. In Bennett's[14] practice, these drugs proved disappointing in both ambulatory and hospitalized patients.

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* The chlorpromazine used in this study was supplied by the Smith, Kline & French Laboratories, Philadelphia, Pa., under the trade name of Thorazine.

Comparative efficacy: reserpine versus chlorpromazine. It is generally agreed that for the most part chlorpromazine and reserpine influence essentially the same types of psychiatric illness. There is, however, a paucity of data that would permit a comparison of the clinical efficacy of these 2 compounds in the treatment of mental disorders of psychotic proportions. Although the importance of such a survey may be vitiated by the fact that the opinions are based largely upon clinical impressions rather than upon well-controlled experimentation, it is noteworthy that, of those who rendered an opinion, 19 of 22 respondents felt that chlorpromazine was a superior drug. It is also of interest to note that Azima's report[15] on the International Colloquium on chlorpromazine held in Paris, France, October 20 to 22, 1955, lists a similar preference for this drug.

Chlorpromazine-reserpine synergism. The studies of Barsa and Kline,[16] Eiber,[17] Tuteur and Lepson,[18] and others kindled hopes that a combination of chlorpromazine and reserpine might prove more potent than either drug alone. To date, this hope remains unrealized, although many investigators (Kugelmass,[19] Holt,[20] Forrer,[7] Kline,[21] Hoch,[22] Overholser,[2] Daugherty[23]) remain convinced that a synergistic effect is obtained by utilizing these drugs in combination. Hollister *et al.*[24] and Kinross-Wright[25] tested sizable groups of patients for a synergistic effect and found none. Others (Freyhan,[26] Ayd,[3] Feldman,[27] O'Neill,[28] Azima,[29] Lehmann[30]), in studying lesser numbers or individual patients, have likewise concluded that the so-called synergism is illusory. Perhaps the strongest indication that the effect is additive is the fact that Tuteur[6] has abandoned to a considerable degree his once very definite ideas to the contrary.

Comparison with other somatic therapies. In spite of the converse opinions previously noted, an overwhelming mass of evidence points to the fact that chlorpromazine and reserpine yield results superior to insulin-coma or electroconvulsive therapy in the treatment of schizophrenia. Except for those previously quoted, my respondents share this impression, but are quick to point out that individual exceptions in which electroconvulsive therapy is far superior are not uncommon. These drugs, especially chlorpromazine, are occasionally effective in the treatment of depressive reactions, but electroconvulsive therap remains the treatment of choice. It is the abuse of these drugs in futile an prolonged attempts to treat depressions that could otherwise be counted upo to respond uniformly and rapidly to electroconvulsive therapy, that wrenche a note of protest from Gahagan[31] and evokes critical comments from other The use of these drugs has brought about a near cessation of the practice c lobotomy, and almost every report on their use in a chronic schizophreni population contains evidence of their superiority over psychosurgery. It i becoming increasingly apparent, however, as has been pointed out by Ayd,[3] that their lack of effect in chronic "EST-fast" depressive reactions, in classica obsessive-compulsive reactions, and in "border-line" states such as pseudo neurotic schizophrenia leaves a sizable group of patients who can be helpe only by lobotomy.

Combined electroconvulsive-ataraxic therapy. There is an admixture of opinio regarding the safety of combining electroconvulsive therapy with reserpin or chlorpromazine. Among those who have reported adverse effects wit reserpine-ECT combinations have been Foster and Gayle,[33] 1 death and severe reactions; Kalinowsky,[34] several severe reactions; Dickel[13] and Shanklin,[1] 1 death and several severe reactions; Bennett,[14] 1 death; Noce,[5] several sever reactions; Azima,[29] 1 death (insulin coma and not ECT); and Epstein,[35] on near fatality. On the other hand, no adverse reactions have been encountere by Bower[36] in treating 30 patients; by Azima[29] in 200 patients; by Freed[37] i 100 patients; or by Forrer[7] (75 per cent of whose patients receive combine therapy), Kinross-Wright,[25] Tuteur,[6] Feldman,[27] Guzvich,[38] Waggoner,[39] an others. I have been consulted directly or indirectly regarding 5 electroconvulsive therapy deaths in the Bay area of San Francisco, Calif., over the pas year. In 3 of these the patients were also receiving reserpine, but I could no be at all sure that this was an important factor.

Those who have reported difficulties with combined chlorpromazine-ECT include Weiss,[40] a severe hypotensive reaction; Epstein,[35] 3 deaths (reported by colleagues) and several severe reactions; Pokorny,[41] 2 deaths that he feels were unrelated to the drugs; Kalinowsky,[34] 1 death reported by a colleague; and Hohman,[9] 2 deaths reported by a colleague. In contrast, the following investigators report considerable experience with this combination without notable difficulties: Solomon,[42] 35 cases; Azima,[29] 300 cases; Steckler,[83] 50 cases; Freed,[37] 100 cases; Ayd,[43] 75 cases and 525 treatments; Overholser,[2] 20 cases; Galvin,[44] 150 treatments; Ford,[1] who describes extensive experience; Kinross-Wright,[25] who has used high doses in many cases for 2 years; Lehmann,[45] who has used this combination 2 years and has also utilized chlorpromazine with insulin-coma therapy; Forrer,[7] 75 per cent of whose patients receive either chlorpromazine or reserpine with ECT; Shanklin,[12] and numerous others. Among others, Shanklin[12] and Ayd[43] feel that this combination speeds recovery, and the latter has presented evidence that it also reduces postconvulsive excitement. Those who combine ECT with ataraxics advise deferring medication until at least 8 hours after giving the medication.

Inasmuch as many of the adverse reports are based upon personal communication it is quite possible that some overlapping has occurred, and it may also be that the frequency of such reports may have been augmented by rumor. On the other hand it is also likely that other cases have gone unreported. Nevertheless, the very fact that fatalities have occurred should serve to warn against the combination of these drugs with electroconvulsive therapy unless there are clear-cut indications that therapeutic results are enhanced by doing so.

Chlorpromazine jaundice: safety of retreatment. Jaundice as a complication of chlorpromazine treatment has been accorded wide attention and must be considered seriously in administration of this drug. In reviewing the literature, however, one is struck by scattered and isolated observations indicating that this drug can be readministered without complication in patients who once suffered from chlorpromazine-induced jaundice. The cases that have reached my attention and the personal experience of my respondents in this connection are included in TABLE 1, from which it can be gathered that, even if the ambiguous terms such as "several," "many," or "a few" are conservatively understated as "2" each, a total of 68 of 77 such cases have been retreated without recurrence of jaundice. One gains the impression that those patients who suffered an acute febrile episode followed by jaundice are more likely to redevelop this complication than are those in whom the onset was rather silent. It is noteworthy that Daugherty[23] and Feldman[27] no longer discontinue treatment with the onset of this complication.

Chlorpromazine dosage. While chlorpromazine dosage has been fairly well standardized to levels of from 40 to 400 mg. daily for neurotic conditions, no uniformity of approach exists regarding dosage for psychotic reactions. Cohen[78] has set down the principle of increasing the dosage until the symptoms are controlled or until signs of toxicity have developed. Various proponents of the "as vigorous as is necessary" chlorpromazine therapy have subscribed to this principle, to which has been added the policy of routine co-administration of ancillary drugs to prevent the occurrence of toxic side effects. Among my respondents who might be considered members of this school are Kinross-Wright[25] (who now uses an average maximum dose of 2000 mg. daily), Ford's[1] group, and Ayd.[53] In contrast, others among my respondents have pointed out that their results have not been enhanced by increasing dosage beyond a set level. Bower[36] sees little advantage in using over 200 mg.; Feldman,[27] 250 mg.; Denber,[4] 300 mg.; Azima,[29] O'Neill,[28] and Waggoner,[39] 600 mg.; Kurland[47] and Pollack,[48] 800 mg.; and Hollister,[46] Overholser,[2] and Galvin,[44] 1000 mg.

My own series includes good responses at relatively low doses of 300 to 500 mg. These results were not improved upon in the same patient retreated with much higher dosage after relapsing. In several instances in which a lower dose of 300 to 600 mg. daily for 1 to 4 months produced unsatisfactory results, however, an increase in dosage to 1000 to 2000 mg. produced a materially better response. It is generally conceded that chlorpromazine offers little help for nonexcited, hebetudinous, anergic, compliant paranoids, yet several such patients maintained on a dosage of 2000 to 2500 mg. for 4 to 6 weeks obtained a surprisingly good response. No comparable group treated with lower dosage

Table 1

Data on the Retreatment of Patients with Chlorpromazine-Induced Jaundi

Investigator	Number of cases retreated with chlorpromazine		Remarks
	Without recurrence	With recurrence	
Denber[4]	Several		
Solomon[42]		1	Fever and jaundice recurred
Azima[29]	1		
Forrer[7]	Several		
O'Neill[28]	Several		
Hollister[46]	2	1	Treatment continued in 1 case, 1 case r curred the first time but not with t second retreatment
Goldman[81]	4		
Daugherty[23]	Several		No longer discontinues treatment
Feldman[27]	Many		No longer discontinues treatment
Kurland[47]	2		
Tuteur[6]	A few		
Pollack[48]	33	2	
Lehmann[30]	2	1	
Elkes and Elkes[49]	1		
Garmany *et al.*[50]	2	1	Pyrexia recurred in 1 of 3
Litteral[51]	1		
Bennett[14]	1		
Ayd[3]	1		Considers retreatment safe
Kinross-Wright[25]	1		Considers danger overemphasized
Borrus[52]	1		
Ford[1]	Some	1	
Margolis	2	1	Pyrexia recurred in 1

is available for comparison, however. Routine co-administration of amphetamine and antiparkinson agents nullified the somnolence, lethargy, and extrapyramidal symptoms that would otherwise preclude such vigorous treatmen

An interesting phenomenon not infrequently observed has been that of standstill course suddenly turning for the better and fixed delusions bein abandoned coincident with the abrupt lowering of dosage to a moderate leve from the high level at which it had been maintained for weeks.

Depressions attributed to reserpine. Depressions associated with reserpin therapy have been the subject of numerous reports, but detailed psychiatri studies are lacking. Those encountered in the literature and those reporte to me in response to my questions are tabulated in TABLE 2.

These depressive reactions occur frequently enough to merit careful scrutin and to serve as a warning against the indiscriminate use of this drug in patient who are unsupervised and prone to depression. One should not deter the us of this valuable agent when valid therapeutic indications exist. All depressions associated with the use of the drug are not attributable, however, to it influence. In a yet unpublished study,[84] Neal and Margolis report on a serie of 17 patients (approximately 10 per cent of the patients applying for treatment at The Langley Porter Clinic over a stated period) who presented a combination of depression and reserpine intake, but in whom many other factor assumed equal importance.

TABLE 2

REPORTS OF DEPRESSION ASSOCIATED WITH RESERPINE THERAPY

Author	Cases	
reis[54]	5	1 required EST*
uller *et al.*[55]	7	5 required EST
chroeder and Perry[56]	5	2 required EST
chor *et al.*[57]	15	2 required EST—1 suicide
ass and Brown[82]	3	
Correspondents		
hanklin[12]	11	
ohman[9]	10	
zima[29]	9	4 attributed to drug
ickel[13]	30	
eldman[27]	2.2 % of cases	
yd[3]	55	Plans report
inross-Wright[25]	15	Plus many mild ones
eal and Margolis[84]	17	Many factors involved

* Electroshock therapy.

Clinical Application

Chlorpromazine. It is no longer possible to present a concise summary on lorpromazine. The clinical effects of this compound are well known, but its ode and site of action are not yet fully understood. Experimental and clinical ata suggest that chlorpromazine acts upon the hypothalamus, which regulates utonomic responses and affective discharges; upon various components of e mesodiencephalic activating system, which is responsible for the control of akefulness, attention, and motor initiative; and that it also has a peripheral utonomic effect. Along with reserpine it possess the unique capacity to tranuilize without depressing, clouding consciousness, or removing inhibitions, d therein lies its advantage over the traditional sedatives in application to most the entire spectrum of clinical psychiatry and medicine.

The clinical application of chlorpromazine is attended by important and metimes dangerous reactions, and it should not be administered indiscrimiately. These reactions are by now a familiar story and need not be described detail here. Precautions must be taken to avoid those reactions due to cal tissue irritation; postural hypotension; potentiation of the depressive acon of narcotics, sedatives, or alcohol; the unusual sensitivity of debilitated aged patients; photosensitivity; and intolerance to heat. As with hyponsive responses, constipation is best handled by vigilant prophylaxis. Other mptoms due to autonomic disturbance are treated symptomatically (that is, asoconstrictors for nasal congestion) or managed expectantly. Blurring of ision is apt to persist during treatment but, as tolerance develops, the sympms of dry mouth, dizziness, and urinary frequency fade, although persistent ypotensive symptoms sometimes necessitate support with ephedrine or mphetamine. Occasionally paradoxical nausea and diarrhea develop and quire reduction in dosage, or anticholinergic compounds or procaine tablets.

The latter are also used for those patients whose retrosternal burning is n relieved by giving the drug with meals, with milk, or with antacids. Par doxical hypertension occurs but is never a problem. Both hypothermia a hyperthermia are encountered but are unimportant, except, that the latter to be distinguished from the "grippelike" syndromes that may presage a sen tivity reaction. Bulimia, weight gain, lack of drive, and somnolence are oft desirable but, if excessive, can be managed by amphetamine compounds or t newer stimulants. Breast engorgement and secretion, menstrual irregularit and increased sexual drive have been noted but offer no problems in manag ment.

Sensitivity reactions involving the liver have already been touched upon a do not necessarily constitute a bar to further therapy. Jaundice has bee observed to fade in spite of continuation of therapy and, in a large majority cases in which treatment is interrupted and readministered, it does not recu Allergic dermatitis only occasionally causes concern. It almost invariab clears without necessitating interruption of therapy and does not recur wit readministration of treatment in those patients whose treatment was on interrupted because of it. In spite of the fact that it occurs in but 1 of 50,0 to 100,000 patients,[59] the possibility of agranulocytosis can never be dismisse

Convulsions are far from uncommon in intensive chlorpromazine therap but they can be readily controlled with a temporary reduction in dosage an the addition of anticonvulsants. Dissociative reactions in neurotics ofte preclude chlorpromazine therapy, especially in those patients whose defens take the form of bizarre somatic symptoms bordering on delusions and in thos with obsessive-compulsive defenses. An organic psychosis that takes the for of restless, confused, searching behavior associated with visual hallucinatio calls for a sharp reduction of dosage or discontinuation of therapy, whereupo recovery is prompt. Extrapyramidal manifestations respond quickly to ant parkinson compounds.

Thus any or all of the complex of symptoms that have led to the term "chlorpromazine (or reserpine) zombie" can be forestalled and counteracted by th co-administration of appropriate ancillary medications. Experience has taugh that in many patients the dosage necessary to effect rapid and maximum control and improvement cannot be reached without producing distressing an incapacitating somnolence, lethargy, and extrapyramidal dysfunction. Thes same symptoms are also prominent in long-term, low-dose therapy. It is in th management of this aspect of treatment and in the growing recognition of th concept that the "chlorpromazine zombie" need no longer exist that I feel th most important advances in the field of psychopharmacotherapy in the pas year have been made.

Reserpine. As with chlorpromazine, the modus operandi of reserpine is n entirely clear. Taking into account the fact that chlorpromazine has stron atropinelike activity, whereas reserpine has no appreciable peripheral actio the physiological response to both drugs is not too dissimilar. Experiment data, however, provide evidence that reserpine possesses an important centr stimulatory function, whereas chlorpromazine acts more by central depressio The clinical indications for both drugs are much the same.

In speaking of the clinical application of this drug, I must lean heavily upon the work of Kline[58, 59, 60, 61] and Noce [62, 63] and their co-workers, who have nitiated use of this drug in the United States. Side effects encountered during reserpine therapy include drowsiness, fatigue, nasal congestion, dizziness, abdominal cramps, diarrhea, urinary frequency, bradycardia, increased salivation, paresthesiae of the extremities, vivid dreams, and edema of the face and extremities. Increased appetite and weight gain are noted. The various components of a Parkinson's syndrome supervene with high dosages and prolonged therapy. Convulsions occur in an appreciable number of patients. Signs of overdosage include anorexia and weight loss, and the development of an organic psychosis. Some patients experience extreme restlessness instead of relaxation. Some develop pseudochills during the early stages of parenteral therapy, and a few cannot tolerate parenteral administration because of intense vertigo. Toxic reactions upon the hematopoetic system or liver have not been observed. Occasionally perforation of a hitherto unrecognized gastric ulcer occurs. This drug has the capacity, as has chlorpromazine, to mask dangerous systemic llness, and for this reason especially careful supervision is necessary in the aged or debilitated. Kline was one of the earlier proponents of the use of ancillary medications such as amphetamine compounds, anticholinergic substances, and antiparkinson agents to control the side reactions of reserpine therapy.

Although dosage must be individualized, the dosage schedule worked out by Kline and Stanley[61] is calculated to extract the full potential of the drug in the reatment of most psychotic reactions. This technique takes cognizance of a "sedative" period of 3 to 10 days' duration, a "turbulent" stage that may last rom 1 to 21 days, followed by an "integrative" period of indefinite duration. Kline uses a daily oral dosage of 3 mg. combined with 5 to 10 mg. parenterally, he latter being adjusted to the patient's tolerance and clinical response. Kline eels that using a dosage in this range enables those patients who are destined o respond to reserpine to do so within a period of 6 weeks.

Many therapists have used much lower dosage in psychotics. For the most part, neurotics have been treated with a daily dosage of under 2 mg., and hyper-ensives with 0.3 to 0.5 mg. The depressive responses encountered in the latter group of patients have already been commented upon. Borderline psychotics or obsessive-compulsive individuals given reserpine may also develop anxiety and dissociative symptoms, as has been noted with chlorpromazine.

Azacyclonol. Azacyclonol, which has been shown by Fabing [64] to have the property of blocking the psychotomimetic effects of LSD and mescaline, has been demonstrated by Fabing and Hawkins[65] and by Proctor[66] to be effective n dispelling symptoms of acute schizophrenia and toxiconfusional states associated with alcohol or surgery. Its therapeutic scope has not been considered o encompass more chronic conditions, although Rinaldi *et al.*,[67] in a double-blind study, obtained behavioral improvement in a small group of chronically disturbed psychotics. The chief advantage of this agent has been its freedom rom toxic side effects.

The problem of evaluating a drug in labile conditions subject to spontaneous emissions is fraught with difficulty. My respondents obtained the expected elief in using azacylconol in acute alcoholic hallucinosis, but even here they were unsure of its role. Hohman,[9] Freyhan,[26] Shanklin,[12] Forrer,[7] Clark, Freed,[37] Pokorny,[41] and Ford[1] found it disappointing. Lehmann[30] in 40 cases Kinross-Wright[25] in 40 cases, and Ayd[3] in 100 cases obtained little respons except in a few toxic states. It is of interest to note that Ayd[69] found it effective in 2 cases of chlorpromazine-induced psychosis, whereas he reports tha Kinross-Wright felt it to be of help in 1 of 2 such cases, and I have used it wit only questionable success in 1 of 2 cases.

Meprobamate. According to the earlier reports of Selling,[70] Borrus,[71] an Lemere,[72] a stride in the direction toward a nontoxic, nonaddicting, nondepressant relaxant was taken with the introduction of meprobamate. Berger[73] ha shown that this drug exerts a depressant action on multineuronal reflexes without significantly affecting those reflexes dependent upon monosynaptic transmission, and it has no important autonomic effects. Side effects are minimal with malaise, excessive drowsiness, dizziness, and ataxia occurring only wit extremes in dosage. The usual dose varies from 3 to 8 or more 400-mg. tablet daily. Favorable results in terms of relief or reduction of insomnia, anxiety headaches, muscle tension, postalcoholic states, and a variety of psychoneuroti symptoms have been reported, and it has been felt that the drug fosters psychotherapy. Sensitivity reactions in the form of urticaria, angioneurotic edema, bronchospasm, fever, chills, syncope, convulsions, and purpura have been encountered, and dependency symptoms have been produced.

As in the case of the other drugs this agent has not met with unqualified acceptance. Several of my respondents have found its effects unconvincing or unimpressive (Hohman,[9] Hoch[22]), except insofar as muscle tension is concerned (Kinross-Wright[25]), or myoclonic epilepsy and cerebral palsy (Ayd[3]). Others (Waggoner,[39] Bower,[36] Freed,[37] and Ford[1] have found it a useful sedative. Pokorny[41] has obtained promising results in anxiety states and in a few paranoid reactions and aggressive personality disorders. On the basis of experience with a limited number of cases, Steckler[83] judges it to be helpful in treating the bizarre somatic delusions of ambulatory schizophrenics.

Lysergic acid diethylamide. Deserving of more comment than can be made in a condensed review such as this is Sandison's[74, 75] utilization of LSD as an adjunct to psychotherapy. Perhaps the greatest significance of this work lies in the fact that it offers an approach to the severe obsessional cases that are so notoriously difficult to treat by other methods. Busch and Johnson[76] also report increased accessibility and encouraging therapeutic gains. A similar approach using mescaline and chlorpromazine has been described by Denber and Merlis.[77]

Other drugs. This section would not be complete without a word regarding the other drugs that have a useful place in psychiatry. Various barbiturates and amphetamine derivatives provide symptomatic relief of agitated or depressive aspects of certain illnesses, but their action is decidedly limited and insufficiently free of side effects to make their prolonged administration desirable. Amobarbital sodium (sodium amytal) among the barbiturates and desoxyephedrine (Methedrine) among the stimulants are drugs of choice for alterng accessibility for diagnostic or therapeutic purposes. The newer stimulant, ipradrol, has not lived up to its promise of being an effective antidepressant vithout significant amphetaminelike action.

Clinical Observations

Observations derived from the use of chlorpromazine in a limited number of atients treated at The Langley Porter Clinic permit no sweeping conclusions, ut they do give support to a conservatively enthusiastic and realistic accept-nce of the value and limitations of this drug in various psychiatric and mixed rganic and functional disorders. This can best be illustrated by the follow-ng tables and charts, in which there is considerable overlap in clinical material, nd from which statistics regarding complications are omitted. Inasmuch as hese data deal with only 131 patients treated over a 2-year period, it is felt hat little purpose would be served in complicating it with an elaborate statis-ical breakdown.

Therapeutic results in this series are classified along a scale ranging from no ignificant improvement to apparent recovery. Regardless of the degree and eeming permanency of remission, patients who are free of symptoms, who ave reached their premorbid states, who are looked upon by their family and ssociates as "cured," and who have resumed their former lives, are classified nder the term of "social recovery." I have learned to distrust the term cure n such severely ill patients so quickly restored to "normality" and, for the sake f clarity and brevity, I shall avoid using "cure" here. Those patients who etain only remnants of their illness and who are able to lead relatively normal ives are classed as "markedly improved." Any lesser degree of response is escribed as "slightly improved" or "unimproved."

TABLE 3 summarizes the results of chlorpromazine therapy given in lieu of lectroconvulsive therapy in conditions other than depressive reactions. hese 47 patients included 40 schizophrenics, 3 patients with involutional sychotic reactions, and 4 patients with hypomanic manic-depressive reactions. wenty-two had had electroconvulsive therapy with previous hospitalizations, nd 5 had received from 10 to 42 treatments immediately prior to hospitaliza-ion at The Langley Porter Clinic. Most of these patients received chlorpro-nazine within the first 2 months of their hospitalization here, but in 6 cases he treatment was delayed from 3 to 6 months.

It can be seen in this table that social recovery took place in 38 (80 per cent), narked improvement in 4 (9 per cent), slight improvement in 3 (7 per cent), nd no improvement in 2 (4 per cent). Of the 38 patients with a social re-overy, 8 (21 per cent) relapsed at various periods after discontinuation of herapy. Four of these were again given chlorpromazine and in each case the revious degree of improvement was achieved. In an as yet undetermined umber, but still a minority, of the socially recovered group and in all of those vith lesser degrees of improvement, maintenance therapy was found necessary r was indicated.

Because of the impracticability of attaching statistical meaning to such a

TABLE 3
RESULTS OF CHLORPROMAZINE THERAPY IN 47 ELECTROCONVULSIVE-THERAPY CANDIDATES*

Results	Number of cases	Per cent	Remarks
Social recovery	38	80	Relapse in 8 (21 %). Chlorpromazine repr duced social recovery in each of the 4 patient retreated. The majority have not require maintenance therapy.
Marked improvement	4	9	All required maintenance therapy.
Slight improvement	3	7	Chlorpromazine therapy was of little value.
No improvement	2	4	

* Exclusive of depressive reactions.

TABLE 4
RESULTS OF CHLORPROMAZINE THERAPY IN 62 SCHIZOPHRENIC PATIENTS

Results	Number of cases	Per cent	Remarks
Social recovery	37	60	Relapse in 10 (27 %). Chlorpromazine repro duced social recovery in each of the 7 patient retreated. The majority have not require maintenance therapy.
Marked improvement	14	22	Maintenance therapy was usually required.
Slight improvement	8	13	Chlorpromazine therapy was of little value.
No improvement	3	5	

small sample, results in the schizophrenic patients are analyzed as a group and not according to clinical categories (TABLE 4). In this table it can be seen that social recovery was achieved in 27 (60 per cent) of the cases, a markedly improved status in 14 (22 per cent), slight improvement in 8 (13 per cent), and no improvement in 3 (5 per cent). Of those who reached a state of social recovery, relapses occurred in 10 (27 per cent). Readministration of chlorpromazine reproduced social recovery in each of the 7 cases in which this was done. A majority of socially recovered patients have not required maintenance therapy, but whether this will be true with a more prolonged follow-up study remains to be seen. It was felt that maintenance therapy was indicated in most patients in the markedly improved class.

FIGURE 1 shows the clinical data on 16 schizophrenic and 1 hypomanic manic-depressive patient treated with chlorpromazine only after failing to respond to intensive inpatient care administered in an elaborate treatment setting. This figure deals only with the patient's care during his stay at The Langley Porter Clinic and offers some degree of comparison between the effects of chlorpromazine and the more traditional treatment methods administered in the sam clinical setting.

Five of these patients received 18 to 46 administrations of ECT, 4 receive full courses of 49 to 50 insulin-coma treatments, 7 were given insulin-com

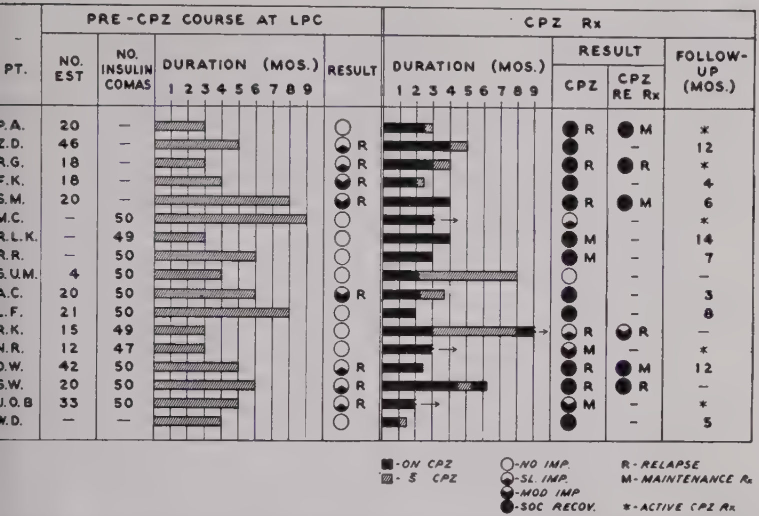

FIGURE 1. Clinical data on 17 psychotic patients (16 schizophrenic and 1 hypomanic manic-depressive) eated with chlorpromazine after unsatisfactory response to intensive inpatient treatment at The Langley rter Clinic.

ierapy combined with 12 to 42 administrations of ECT, and 1 received no matic therapy. Their hospital stays ranged from 3 to 9 months, during hich time the efforts of a well-equipped and relatively completely staffed eatment team were directed in their behalf. In 14 of these 17 cases the reılts of chlorpromazine therapy exceeded those produced by the conventional ierapies, and the remaining 3 failed to respond to either treatment.

Patient Z.D. provided an example of a decidedly favorable chlorpromazine fect. She had relapsed repeatedly during 5 months of hospitalization, during hich period she had received 46 treatments of ECT. Her acute and florid unfferentiated schizophrenic symptomatology then vanished during a 4-month ourse of chlorpromazine, and she has remained well for over a year after disontinuance of the drug.

A good response but one of shorter duration is illustrated in FIGURE 2. This atient (R.G.) adjusted at what her family considered to be a near normal vel prior to, between, and after schizophrenic episodes in 1949 and 1952. ı her third attack she failed to respond to 18 treatments of ECT and then ached a state of apparent remission while treated for 3 months with 300 mg. aily of chlorpromazine. After relapsing she was treated with a much higher osage of chlorpromazine, receiving as much as 2400 mg. daily during the first onth of treatment. Hopes that this would result in a more sustained remison were not realized, as she again relapsed a few months after discontinuing ie drug. She is now receiving chlorpromazine at home, and there are hopes at rehospitalization will be avoided.

The course of another schizophrenic patient (M.B.) with recurrent episodes

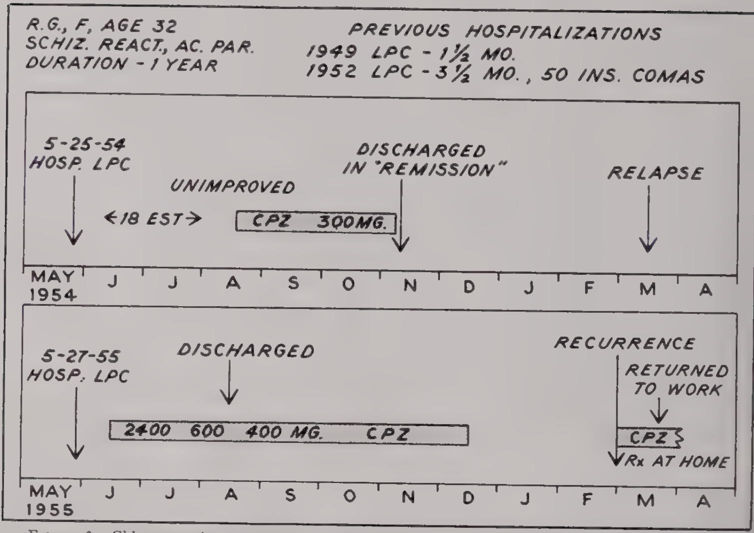

FIGURE 2. Chlorpromazine response of a patient with episodic exacerbations of a paranoid reaction.

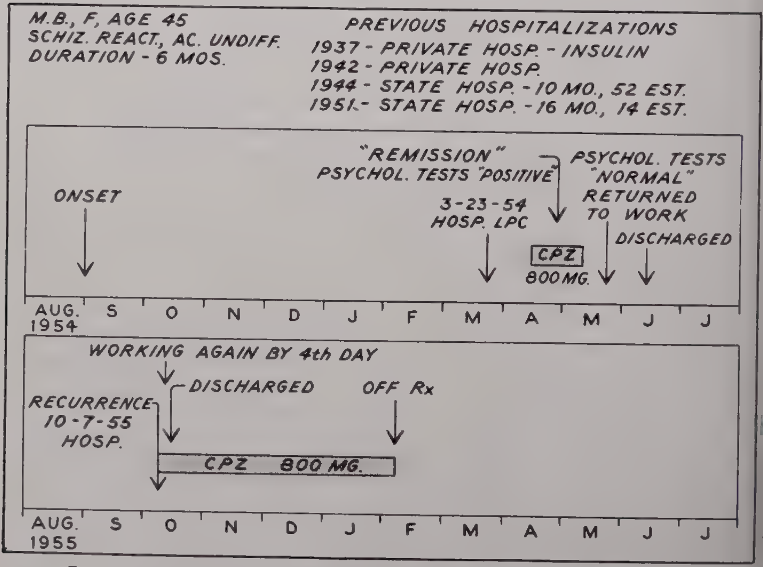

FIGURE 3. Response to chlorpromazine of a patient with recurrent schizophrenic attacks.

d a similarly good but ill-sustained response to chlorpromazine is illustrated FIGURE 3. This 45-year-old medical secretary had suffered schizophrenic isodes requiring prolonged hospitalization and somatic therapy on 4 occaons between 1937 and 1951. With her next attack, in 1954, she was hospilized for 1 month without improvement, after which she responded promptly a short course of chlorpromazine and was able to return to work within 5 eeks. She relapsed in 5 months, and prompt resumption of chlorpromazine ermitted her return to work within 4 days.

Another patient, A. O. C. (FIGURE 4), a chronic paranoid schizophrenic, obined a state of apparent remission on chlorpromazine but quickly relapsed 2 occasions when the drug was discontinued. On each occasion she reported e return of hallucinations and asked that therapy be resumed. In contrast ith the previous 2 patients she is more than willing to continue taking the lorpromazine as prophylaxis against a relapse.

In order to avoid creating the impression that such prompt relapses are charcteristic of chlorpromazine therapy and not of other therapies, the case record f patient B. W. is presented in FIGURE 5. Schizo-affective episodes requiring matic therapy and moderately lengthy hospitalizations occurred in 1948, 950, and 1951 and, in the intervals between these attacks, this patient received sychotherapy. With her fourth attack, in 1954, she made a partial response an abbreviated course of chlorpromazine and then remained hospitalized r the remainder of the year as she "worked through" another attack with total push" and intensive psychotherapy, without drug or somatic therapy. uring the next year she was seen in outpatient therapy but again relapsed fter discontinuing her "regular but reluctant" attendance.

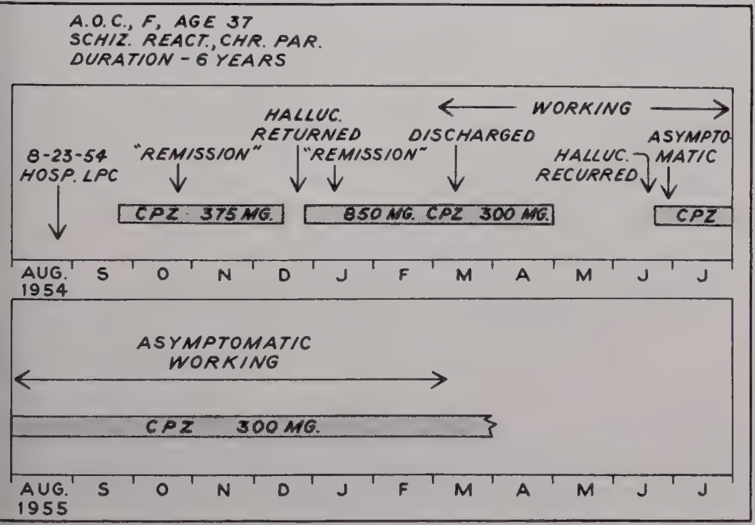

FIGURE 4. Response to chlorpromazine of a chronic paranoid schizophrenic.

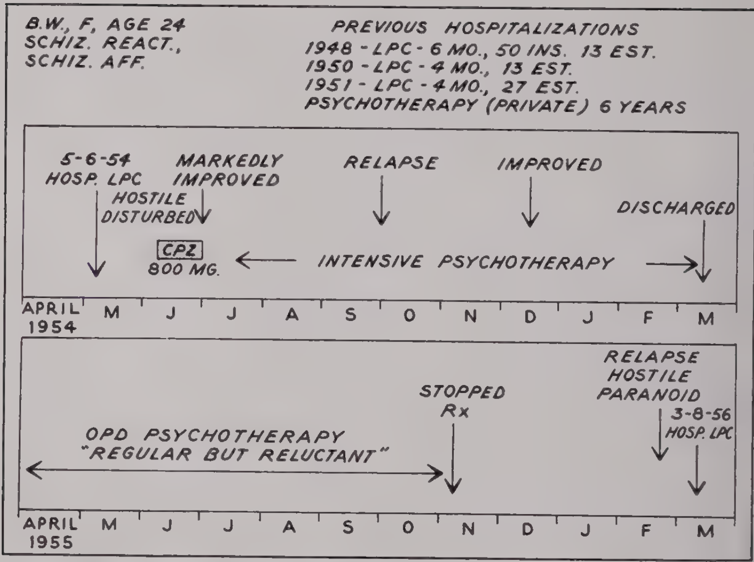

FIGURE 5. Clinical course of a recurrent schizo-affective reaction treated principally with intensive psychotherapy.

In many of the more difficult and floridly psychotic patients in this group results seemed contingent upon the employment of what would be considered by some standards to be rather heroic chlorpromazine dosage. Even with the utilization of an "as vigorous as necessary" approach, however, there seemed to be little advantage in exceeding daily dosage levels of from 2000 to 2500 mg. It should be pointed out that in order to maintain a clinically desirable state of improvement a few patients required daily "maintenance" dosage of 600 to 1000 mg., which is higher than the maximum dosage recommended by numerous investigators. An example of such a patient is shown in FIGURE 6, which is borrowed and slightly modified from a previous report,[79] and in FIGURE 7.

This patient (D. W.), with an acute, undifferentiated schizophrenic reaction, failed to respond to 5 months of hospitalization, 42 ECT and 50 insulin-coma treatments. On 500 mg. daily of chlorpromazine she was able to leave the hospital, but she made an unstable adjustment for 5 months while being carried on this and higher levels as an outpatient. After attempting suicide when taken off of the drug, she was rehospitalized and, for the next 3 months, an intensive effort at psychotherapy was made. This proved fruitless, and she was then treated with levels of chlorpromazine up to 2500 mg. daily, which resulted in a prompt disappearance of her symptoms. She is now adjusting well at home on a 600-mg. daily dosage.

Such experiences as these lend themselves to various interpretations. It can

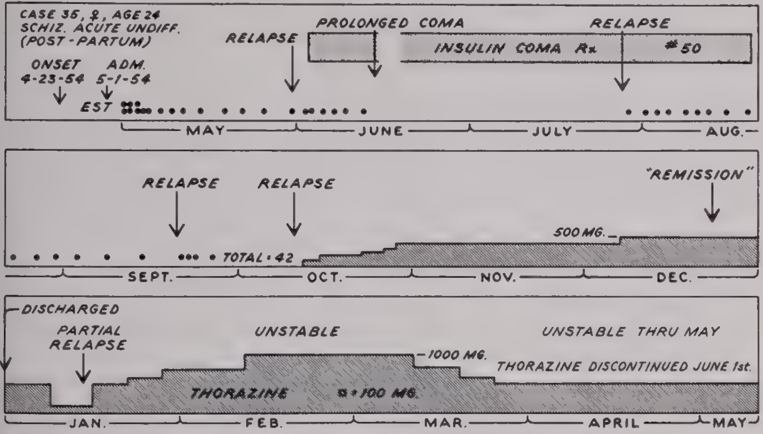

FIGURE 6. Clinical course of a schizophrenic patient (D. W.) treated for a prolonged period with Thorazine in low dosage. Social recovery was obtained, but there was no protection against relapse when the dosage was lowered or when stress was increased.

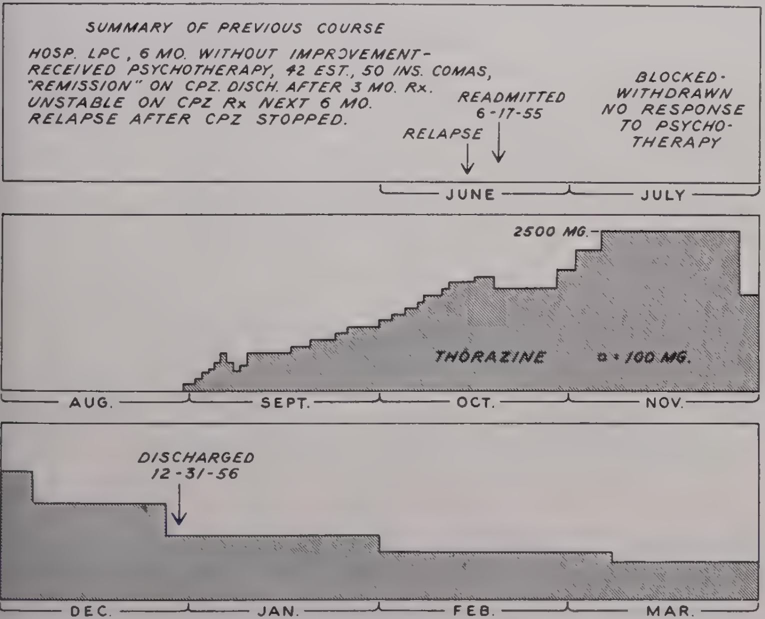

FIGURE 7. Continuation of the clinical course of case No. 35 (FIGURE 6): patient D. W., with an acute, undifferentiated schizophrenic reaction.

be seen that in some patients chlorpromazine exerts only a "sedative" effec regardless of dosage. In others "its beneficial effect lasts little beyond th period of administration of the drug." In many cases, if the compound i administered in inadequate amounts, "its effect is next to useless." Even whe a prompt disappearance of refractory psychotic manifestations occurs wit the administration of chlorpromazine, there have been striking differences o opinion regarding the cause and nature of the remission. An example of thi is provided by the case of patient W.D. (FIGURE 1), who reached a premorbi state of adjustment during 1 month of chlorpromazine therapy after remainin delusional and withdrawn for 4 months in spite of the vigorous efforts of he therapist and the hospital staff. No one can gainsay the importance of psychotherapy in this patient now and, perhaps, for the rest of her life but, by the sam token, the influence of chlorpromazine cannot be discounted. For example in following 1 psychotic patient over the course of 2 years the complacency o three residents, treating this patient in turn, was severely shaken upon seein the behavioral gains and "insight" that each had attributed to his own psychotherapeutic efforts disappear upon the omission of chlorpromazine from the therapeutic program.

Time and space do not permit a detailed presentation of the various othe cases treated in this series. Numbered among these were 13 with depressive reactions in which group social recovery occurred in only 6. Also include were 7 cases with multifaceted complaints best classified under the category o pseudoneurotic schizophrenic reactions in whom the clinical response wa limited indeed. Circumscribed improvement was also obtained in a samplin of 13 patients with organic brain disturbances. Two cases of spasmodi torticollis obtained gratifying reduction in symptomatology, but results wer less impressive in other disorders of movement. Ten patients with intractabl pain were treated. Three with atypical facial neuralgia and 1 with intercosta neuralgia were unimproved. Symptomatic improvement resulted in 2 tabetics and in 1 case with postsympathectomy neuralgia, but the influence of suggestion was difficult to clarify.

Preliminary studies suggest that chlorpromazine has a selective action upon the site of origin of the thalamic pain syndrome. One such patient experienced moderate relief of his burning dysesthesia but no relief from pain originating from a chronic hip disorder. Two other patients with thalamic pain syndromes have also obtained gratifying relief from this drug. Another patient with a similar type of pain from a brainstem lesion experienced little relief and found her associated pyramidal and cerebellar deficit enhanced while taking the drug. One of these cases is described in detail elsewhere.[80]

Summary

A survey of the current opinions and experiences of leading investigators in the field of psychopharmacotherapy regarding the mode of application and the place in psychiatry of the newer tranquilizing agents serves to re-emphasize the diversity of opinion regarding the usefulness of these drugs and the variability of techniques in their utilization. Prevailing trends indicate that

lthough reserpine remains a useful and potent compound, its effects are over-hadowed by those of chlorpromazine. Considerable skepticism exists regard-ng the possibility of synergism between these 2 drugs. The place of azacy-lonol appears to be limited to the short-lasting, toxi-confusional or acute allucinatory states, although it is felt by some that a wider application is ndicated. Opinion is far from uniform, but favorable reviews from various ources affirm the usefulness of meprobamate in tension symptoms. The herapeutic role of the newer stimulants is comparable to that of the older ones vith only questionable reduction in side effects. A compilation of experience egarding the retreatment of patients with chlorpromazine-induced jaundice rovides evidence that a large majority of such patients can be retreated with-ut complications. The depressogenic aspect of reserpine is affirmed by the ersonal experience of numerous investigators. My experience with a small roup of carefully followed cases given intensive chlorpromazine therapy pro-ides grounds for a "conservatively enthusiastic" acceptance of the values and imitations of this drug in various psychiatric and mixed organic and functional isorders. The importance of adequate dosage and the parallel administration f appropriate ancillary drugs in both chlorpromazine and reserpine therapy is tressed.

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Discussion of the Paper

S. Malitz (*Department of Experimental Psychiatry, New York Psychiatric Institute, New York, N. Y.*): I congratulate Lester H. Margolis for his excellent paper. Our own experience with chlorpromazine at the New York Psychiatric Institute extends over a 2½-year period, and our findings parallel his very closely. We first used the German product Megaphen until chlorpromazine was made available in the United States.* Our patients have included predominantly pseudoneurotic schizophrenics, and this may account in some measure for the differences between our findings and others. One third of our group were inpatients, the remainder outpatients. Some of our patients have been maintained on the drug for as long as 2 years without interruption, with no ill effects. There is a marked individual variation among patients in regard to the dosage necessary to produce therapeutic effects and also in regard to the amount of drug that will elicit side reactions. Our maintenance dosages varied between 75 and 2400 mg. The average dosage was between 200 and 400 mg. Some patients developed side reactions on 150 mg. of the drug, and others showed a minimum of side effects on 1000 mg. daily. As a general rule the incidence of side effects increased sharply with dosages above 600 mg. daily.

It was our experience that the more overtly disturbed was the patient, the more effective was the action of chlorpromazine. Calming effects in patients where motor manifestations of anxiety were minimal or absent were far less consistent than in agitated or assaultive patients. This observation transcended diagnostic boundaries. Since most of our patients, both hospitalized and outpatients, fell into the less disturbed group, results with chlorpromazine alone were frequently disappointing. This was especially true with the "garden-variety neurotic," whose predominant symptoms were usually expressed in the form of phobic mechanisms, hypochondria, or obsessive preoccupation.

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* Smith, Kline & French Laboratories, Philadelphia, Pa. (Thorazine); Bayer Co., Leverkusen, Germany, (Megaphen).

In some patients who showed improvement with the drug, relapses occurred both while receiving medication and shortly after its discontinuance. In patients who relapsed while receiving the drug, raising the dosage sometimes resulted in a return of the improvement. In others an adaptation seemed to take place, and no further improvement was forthcoming. Some of these patients, when started on other phenothiazine derivatives without their knowledge, once again improved. One of these derivatives, brom-piperidinochlorphenothiazine, shows great promise. It appears to have an effect equal, therapeutically, to chlorpromazine, while showing fewer side effects. The same compound, without the brom radical, piperidinochlorphenothiazine, had been proving effective until we discovered that one of our patients developed a retinitis pigmentosalike condition with the drug. Fortunately his vision has returned to normal, but the salt-and-pepper pigmentation remains throughout the retina. It has now been more than a year since this patient received the drug. We have since learned of several similar cases in Switzerland.

Another interesting side effect that we saw early in our research on 6 patients and have not seen since was a myesthenia-gravislike reaction consisting of a choking sensation in the throat, difficulty in swallowing and chewing, thickness of the tongue, and slurred speech, with occasional respiratory embarrassment. Fortunately the condition was self-limiting. Response to Prostigmin was equivocal. Since this side effect has not recurred, perhaps it could have been related to the particular batch of chlorpromazine we were using at the time.

After treating patients for 2½ years we feel it is still impossible to predict with any degree of statistical validity how many patients can have the drug discontinued and how many will have to be kept on it indefinitely. We cannot confirm the observations of others that the majority of patients do not need some form of maintenance dosage, even when receiving accompanying psychotherapy.

Our experience with chlorpromazine and reserpine together is similar to that of Doctor Margolis. We found no advantage in their combination. We have found oral chlorpromazine to be more rapid in action and more effective clinically than oral reserpine, in spite of the greater amount of minor side effects occurring with the former. Intramuscular reserpine, while much more delayed in action, compares more favorably with intramuscular chlorpromazine.

The role of chlorpromazine in psychotherapy has not been touched on by Margolis. This is an area very difficult to evaluate because of the many variables, and we are still cautious in arriving at any definite conclusions. There is little doubt, however, that in certain patients with anxiety so marked that they cannot even get to the doctor's office, let alone relate in therapy, the drug can perform a real service in alleviating their anxiety sufficiently to make them more accessible to psychotherapy.

Our findings with chlorpromazine in depressions and with azacyclonol have been disappointing and similar to those reported by Margolis.

A REVIEW OF COMPARATIVE BEHAVIORAL PHARMACOLOGY

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Valter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

Recent developments in chemotherapeutic approaches to the problems of linical psychopathology have stimulated renewed interest in laboratory testng methods for evaluating the behavioral and the central nervous system efects of drug administration. Animal-conditioning techniques provide at least ne potentially valuable assessment method, although the history of experinental work in this general area amply attests to the numerous difficulties involved in the selective definition of specific drug activity at the neural and ehavioral levels. The promise of more precise experimental control and perational definition, however, has encouraged a vast comparative psychoharmacological literature over the past 50 years or more, although such exerimental efforts on animals have seldom been systematic, and the results ave almost always been controversial. In addition, the restrictions imposed y available behavioral techniques have severely limited, until only very reently, the scope of such investigations. By and large, comparative laboratory tudies have focused upon drug effects in relation to general activity, learning, onvulsive behavior, and the like, with considerably less emphasis upon the ffects of pharmacologic agents in motivational-emotional response patterns resumably of more immediate relevance to current clinical problems.

As early as 1898, for example, reports had begun to appear on decreases in he general activity level of the laboratory rat following alcohol ingestion,[1] robably the earliest of a long series of studies over the past half century or nore related to the problem of drug effects upon general activity. The well-nown depressing effects of the barbiturates upon such activity-level measures ave received adequate emphasis in this animal experimental literature,[2, 3] and he effects of a large number of stimulants have been studied with respect to uch activity changes.[4-10] Similarly, considerable animal research has been irected toward elucidating the relationships between experimentally induced hemical changes and innate behavioral patterns such as mating, and the like. The critical role of endocrinological preparations in the control of sexual beavior, for example, has been extensively explored at the comparative laboraory level, and the classic work of Beach[11] and others[12-14] typifies the emphasis n this area. Even more recently, however, the effects of a large number of allucinogens, stimulants, anticonvulsants, and depressants have been studied n relation to their effects upon the web-building behavior of spiders.[15]

Of somewhat more direct relevance to current concerns with the relationship etween the nervous-system effects of drugs and neuropsychiatric problems vould seem to be the rather extensive comparative laboratory work on conulsive behavior. Investigative efforts concerned with the general problem f drugs and convulsive activity reveal, for the most part, a twofold emphasis. irst, inquiries into the behavioral consequences of convulsion-inducing drug dministration have emphasized problems raised by the clinical application of such methods in psychiatry. In this respect the detrimental effects of i sulin and Metrazol upon the maze habit and conditioned reflex have been e tensively investigated,[16-23] and provocative reports by Gellhorn and his co leagues[24-29] have indicated that such chemically induced convulsions may a tually produce the recovery of extinguished conditioned avoidance behavio Second, the effects of pharmacological agents upon convulsive activity produce experimentally by other forms of central nervous system stimulation hav provided an additional continuing source for animal experimental inquiry int neurological mechanisms of convulsive phenomena in general, and into therapeutic approaches to seizure problems in particular. For the most part, an mal laboratory work on this problem has concentrated on the incidence c audiogenic convulsions in rodents as a function of various drug treatment although some concern with the electroconvulsive shock threshold in this sam respect has also been apparent. A large variety of substances have been explored with respect to their ability to increase or decrease seizure thresholds o susceptibility,[30-73] although controversial and contradictory reports continu to prevent a clear definition of many such drug-activity relationships.

The effects of various drugs upon animal learning and discrimination behavior have also been rather extensively studied by numerous investigator but, again, the influence of particular pharmacological agents has not as yet bee clearly delineated. Innumerable pharmacological and psychological shortcomings have imposed severe limitations upon such studies and, only recently with the development of more refined and sophisticated behavioral technique and the re-emergence of pharmacological interest in chemically induced psychological changes, have these restrictions begun to appear less stringent. Th numerous maze and problem-box studies that have characterized the pas half century of research in this area, however, can be seen to provide the backdrop against which present developments in behavioral pharmacology stand i sharp relief. It has been reported that a wide variety of drugs actively affec even these rather gross measures, although the discriminative capacity of suc techniques for specific drug-behavior relationships certainly leaves much to b desired. Alcohol, for example, reportedly produces uniformly detrimental effects upon maze and conditioned-reflex behavior,[74-80] although a more refine definition of specific time-discrimination effects and the like has required th development of more precise conditioning techniques.[81]

Even more disconcerting, however, have been the many conflicting report upon stimulant drugs (amphetamine, caffeine) in this rather extensive animal learning literature,[9, 82-88] emphasizing again the need for a more precise definition of the behavioral variables under examination.[81] The generally detrimental effects of the opium derivatives and nicotine upon acquisition an learned performance are emphasized even in much of this past work,[89-97] although the barbiturates seem to have received the most attention in this respect, probably because of their wide clinical use as sedatives and hypnotics For the most part, these results also indicate a consistently detrimental effec upon the somewhat restrictive maze and conditioned-reflex measures[88, 98-1 but, again, the more recent development of behavioral-control techniques i

e animal laboratory have begun to make possible the differential analysis of
lective barbiturate effects upon various aspects of a conditioned repertoire.[107]
he bromides,[88, 108] glutamic acid,[109-118] various endocrine preparations,[119-124]
nd the antihistaminics[125] have figured prominently in the half century of
imal experimental work concerned with drug effects upon broadly defined
arning phenomena, but the limitations in behavioral technique combined with
adequate pharmacological control procedures for these drugs, too, have
oduced a variety of nonspecific and, in many instances, frankly contradictory
ports.

Of perhaps somewhat more direct relevance to current neurological and psy-
iatric concerns with pharmacotherapeutic approaches in psychopathology
the rather extensive animal experimental work on "emotional behavior"
nd "conditioned neurosis." Although many interesting and provocative con-
ibutions to the drug-behavior literature have characterized such research
forts, in this case also the breadth and precision of experimental analysis
s been rather obviously limited by the lack of operational definition and
lequate control of the variables under scrutiny. Certainly, recent method-
ogical advances in behavorial conditioning techniques will be seen to con-
ibute significantly in this area also.

As early as 1927, reports from Pavlov's laboratory indicated a concern with
e problem of drug influences and "experimental neurosis."[126] Indeed, this
rly work with bromides effectively set the stage for a rather extensive ex-
oration of conditioned-reflex changes in a complex discrimination situation as
function of a wide variety of drugs, including the barbiturates, alcohol,
yoscine, cortin, epinephrine, and many others.[88, 120, 127-130] The related studies
Masserman and his colleagues,[134] and the many variants of his conditioned
neurotic" reaction patterns (feeding-punishment conflicts) that his work has
spired, have also provided the setting for numerous drug-behavior investiga-
ons related to experimental psychopathology. Starting with morphine, al-
hol and, as well as the barbiturates, many pharmacological agents, including
e anticonvulsants and even some of the newer "tranquilizing" agents, have
en explored with respect to their alleviating effects upon "conditioned neu-
sis" established in this fashion.[94, 131-136] The specifications of behavioral
riables in most of the studies on "conditioned experimental neurosis" leave
uch to be desired, however, and it is difficult to define the critical relationships
tween the numerous pharmacological agents under consideration and the
mplex of behavioral functions that have been identified with much oversim-
ified clinical labels. It is virtually impossible, for example, in most of these
udies, even with the use of such generally complex behavioral situations, to
parate the specific effects of a given drug upon those aspects of the organism's
havioral repertoire presumably concerned with "emotional" or "affective"
sponse patterns from the more nonspecific effects associated with activity
pression, sensory decrements, and the like, known to characterize the action of
any such pharmacological agents. At best, considerable ambiguity charac-
rizes the identification and specification of such behavior descriptions as
xperimental neurosis," "conflict," and "stress," and the need for operational

definitions is imperative if analysis of the relationships between drugs a psychiatrically relevant aspects of behavior is to be made possible.

Several more recent attempts to parcel out the variables that might contri ute to the rather molar "conditioned-neurosis" situation have been conce trated upon somewhat more restricted aspects of emotional-behavioral patter and their relationship to pharmacological agents. Specific attention has be directed to the relatively quantitative aspects of conditioned "avoidance "fear," and similar response patterns, and the beginnings of reliable and oper tionally definable affective behavioral assay techniques appear to be emergi as a result of such efforts.

To date, the conditioned avoidance technique has been the most popul approach to this problem. Several reports indicate, for example, that dru such as physostigmine, tetraethylammonium chloride, mescaline, reserpin pentobarbital sodium, chlorpromazine, and pentylenetetrazol produce a d pressing effect upon a conditioned avoidance response of the barrier-crossir or lever-pressing variety, while amphetamine and adrenocorticotrophic ho mone (ACTH) apparently exercise a somewhat facilitative effect upon suc avoidance behavior.[137-143] Interpreting such drug effects upon instrument avoidance behavior as presumably relevant in any specific sense to the fea or anxiety-reducing properties of these several pharmacologic agents woul appear tenuous, to say the least, however, since few of the studies that aspi to such extensions of the data have used adequate controls for generalized moto effects, sensory decrements, and general malaise. Any or all of these coul produce a depression of avoidance behavior independently of any specifi emotional change.[144] As a matter of fact, some studies utilizing more direc measures of conditioned fear or anxiety relatively uncontaminated by instru mental motor responses have indicated little, if any, effect with such drugs a tetraethylammonium chloride.[144, 145] At least one report,[146] however, suggest that morphine may alter such behavior. The results obtained with recentl developed tranquilizing drugs may also be somewhat more encouraging whe refined behavioral assay methods are employed. Although, to date, few oper ationally quantitative and systematic studies have been made on the specifi behavioral changes associated with these tranquilizers, gross and dramati alterations in the affective response patterns of several animal species, par ticularly the monkey, have been reported following the administration c reserpine,[147-149] and it appears to be merely a matter of time before a complet profile of behavioral responses to such drugs will be defined in the comparativ laboratory, with rapidly emerging conditioning control techniques.[81, 150-152]

Recent Developments

Among the many advances in psychological science made in the past sever years, one group of contributions emerging from animal laboratory approache to the experimental analysis of behavior seems to stand out with particul prominence in considering methodological advances in comparative psych pharmacology. Reference here is made to the development of operant co ditioning techniques in the analysis of behavior and the application of suc methods to a wide variety of experimental problems in comparative and physiological psychology. Within the past few years, Skinner[152] and Ferster[151] have outlined the conceptual framework and basic methodologies involved in this approach and, more recently, the systematic application of these techniques to specific areas of behavioral analysis have been reported.[150] In addition, numerous individual experimental reports in current psychological, physiological, and now even pharmacological journals have begun to indicate the broad range of the investigative areas in which such behavioral control techniques have been ound useful. Morevoer, there is considerable evidence of increased experimental activity (as yet unreported) in several laboratories, with particular mphasis upon the comparative analysis of drug-behavior relationships utilizng operant conditioning techniques.

The basis for this enthusiastic acceptance of the free operant in comparative psychopharmacology is not difficult to discern when consideration is given to he rather pressing need for sensitive and reliable preclinical assay techniques nd the woefully inadequate answer to this problem that is provided by the xtensive literature in the area just all-too-briefly reviewed. Indeed, many of he critical shortcomings that limit the usefulness of the maze technique and onditioned-neurosis situations for the analysis of such drug-behavior relationhips appear less restrictive with operant conditioning methods.

One major advantage of this new method is the fact that a relatively broad pectrum of behavioral-response patterns can be quantitatively and operaionally identified and defined. Simple and complex discriminative capacities re assessable within the limits of an experimental situation that also provides reliable estimate of the general activity level, motor functioning, and sensory ecrements.

FIGURE 1 illustrates the multiple-schedule control of an animal's behavior n such a lever-pressing situation with a monkey working for a sugared orange-uice reward. In this figure the curve represents a cumulative record of the nimal's lever responses throughout a 90-minute experimental session, with he number of bar presses indicated on the ordinate and the time indicated on he abscissa. The animal has been trained to distinguish 3 different colored ghts (these could be any kind of discriminable sensory stimuli) as signals ndicating that a drop of orange juice is obtainable on one or another of 3 lever-ressing reward schedules. In one of these schedules, *A* (fixed ratio), the nimal receives the orange-juice reward only after a fixed number of lever esponses (50) has been emitted, thus differentially reinforcing the high bar-ressing rates shown in the *A* portions of the cumulative curve. On the second chedule, *B* (fixed interval), a lever response produces the orange-juice reward nly after a fixed interval of time (5 minutes) has elapsed since the *last reward*. his contingency is seen to produce the scallop-type curve shown in the *B* ctions of the cumulative record. The animal shows virtually no response in e early portions of the interval and an accelerating rate of bar pressing in the tter part of the period until the first response after the fixed 5-minute interval roduces the reward. Finally, the third schedule, *C* (VI, DRL), differentially inforces low lever-pressing rates by providing for the delivery of the orange-

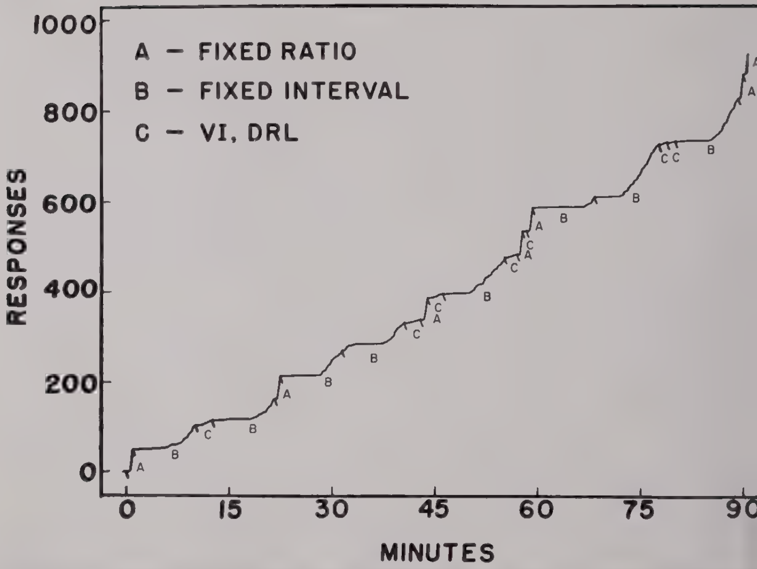

Figure 1. Sample cumulative-response curve, illustrating the performance of a monkey under multipl schedule stimulus control.

juice reward only after an interval of time (30 seconds) has elapsed since th *last response.* The slow, steady response rates shown in the *C* sections of th cumulative record develop when the contingencies of such a schedule reinforc delayed bar pressing. Of course, characteristic response patterns that develo for each of these different reward schedules typify the animal's performanc when each schedule is used alone, and the degree of stimulus control in th multiple-schedule situation is apparent from the elicitation of the appropriat lever-pressing pattern by a specific visual stimulus independently of the orde sequence, or frequency of the stimulus presentation.

A second major advantage of such recently developed behavioral contr techniques is that specific aspects of an organism's behavioral repertoire ca be selectively evaluated and dependably separated within a response patter Emotional or affective reactions can be independently assessed without con plicated and equivocal interpretive problems involving general nonspecifi behavioral and motor disturbances, debilitation, and the like.

Figure 2, for example, illustrates a technique for superimposing a co ditioned emotional response upon a stable lever-pressing habit for a liqu reward. This figure shows the cumulative-response record of a monkey r ceiving repeated pairings of a clicking noise (*cl.*) followed by a brief painf electric shock (*sh.*) to the feet during an extended lever-pressing session for sugared orange-juice reward. Using alternating 5-minute periods of clicki and no clicking, as illustrated, the animal can be seen to develop a strong co

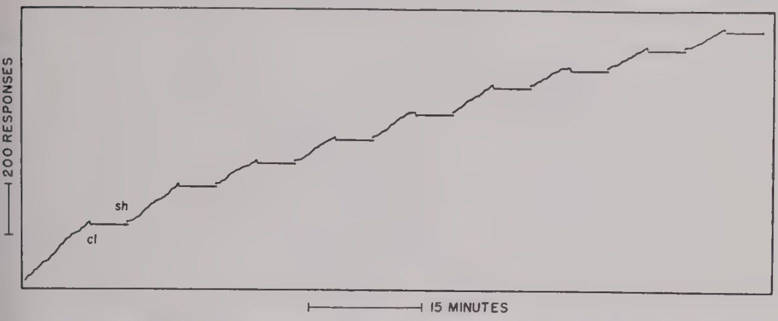

Figure 2. Sample cumulative-response curve, showing the performance of a monkey in an emotional-conditioning situation. The record illustrates the complete suppression of the lever pressing that develops during repeated presentation of the clicking stimulus (indicated by the offset sections of curve *cl.*) terminated contig-uously with the pain shock *sh.*

ditioned emotional response to the presentation of the clicking alone, characterized by a virtually complete cessation of lever pressing, crouching, immobility, defecation, and piloerection. In the 5-minute periods without the clicker, however, no such behavioral disturbance is discernible. The stable, reproducible, durable, and quantifiable properties of such conditioned emotional-behavior patterns would seem to provide an ideal base line for the evaluation of specific drug effects upon affective-response systems, while concurrently controlling more generalized motor disturbances such as malaise.

Finally, it is now quite clear that such operant conditioning techniques are selectively sensitive to a wide variety of experimental operations, including central nervous system damage, electrical stimulation, and pharmacological agents. Alterations in the specific aspects of a behavioral repertoire can be effected differentially with experimental controls inherently present within the remainder of the response pattern.[153-157]

Figure 3, shows the differential effects of 2 drugs, amphetamine and reserpine, upon behavior patterns involving the conditioned emotional response llustrated in figure 2. By comparison with the normal saline record shown n the upper section of figure 3, amphetamine in a dose of 2 mg./kg. can be seen to increase the total lever-pressing output more than 100 per cent during the 1-hour experimental session. This increase in rate, however, is accounted for completely by an increase in the number of responses during the periods between the presentation of the conditioned clicking stimulus and, in fact, the rate is actually observed to *decrease* in the presence of the auditory warning signal. In contrast, the bottom section of the figure shows that 0.2 mg./kg. of reserpine can actually *increase* the rate of response during the emotional-stimulus periods, even though the over-all lever-pressing rate is depressed more han 50 per cent by comparison with the normal saline session. Significantly, n reducing the strength of the conditioned emotional response, this reserpine ffect is definable independently of the suppressing effect of the drug upon the ever-pressing activity of the animal.

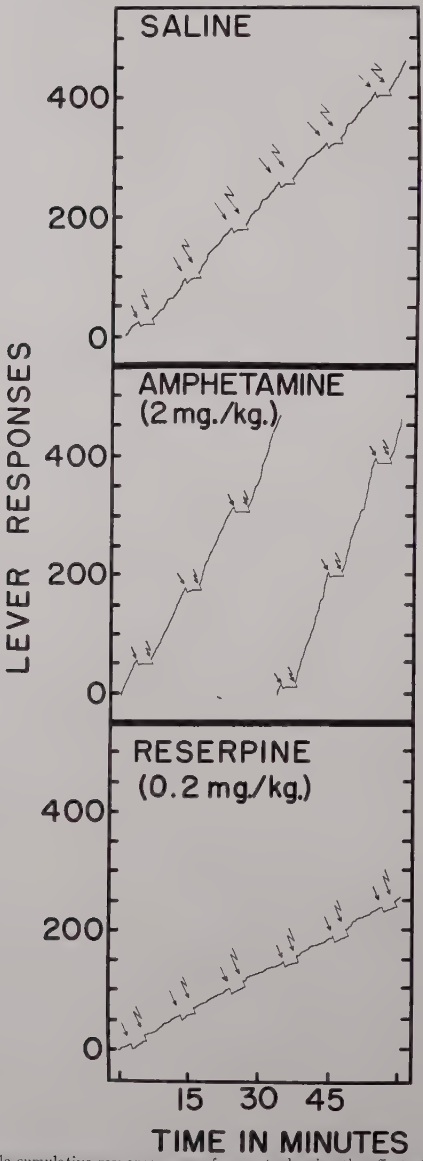

FIGURE 3. Sample cumulative response curves for a rat, showing the effect of amphetamine and reserpine upon lever pressing and upon the conditioned emotional response. The oblique solid arrows indicate the onset of the conditioned auditory stimulus, and the oblique broken arrows indicate the termination of the conditioned stimulus contiguously with the brief unconditioned grid-shock stimulus to the feet.[158]

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Discussion of the Paper

David Lester (*Laboratory of Applied Physiology, Yale University, New Haven, Conn.*): The effects of intraperitoneally administered pentobarbital (5 and 10 mg./kg.) and alcohol (0.5 and 1.0 gm./kg.) have been assessed in albino rats in a situation involving discrimination between the presence or absence of light. The 6 rats used received a reinforcement of water at each 16th bar press. With a rigorous design it was found that both compounds at each dosage level significantly decreased the total responses of the animals. Alcohol, however, did not affect discriminative accuracy, while pentobarbital sodium increased errors from an initial stable level of 2 per cent to 7.5 per cent ($P < 0.05$).

The considerable differences between the experiment cited and that of Blough are difficult to reconcile. It appears that close attention must be given to the species used and the types of scheduling and discrimination employed.

SOME EFFECTS OF DRUGS ON VISUAL DISCRIMINATION IN THE PIGEON

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Joseph V. Brady, elsewhere in these pages, has mentioned some of the operant conditioning techniques that are being developed to assess the behavioral effects of drugs. In this paper I shall describe some preliminary results obtained with still another method of this sort.

Both psychotic behavior and drug-induced behavior are often characterized by a lack of appropriateness to environmental stimuli. To put it in another way, stimuli appear to have less control over behavior than usual. The present method is specifically concerned with the stimulus control of behavior and drug-induced changes in this control. The procedure derives from B. F. Skinner's operant-conditioning methodology. It is rather complex and will be analyzed in detail in a future paper. I can describe it only briefly here.

Procedure

Five experimentally inexperienced male domestic pigeons served as subjects. They were maintained at about 70 to 75 per cent of ad libitum weight during the experiment.

Each pigeon worked in an insulated subject chamber. Its task was a so-called "conditional" discrimination. The bird confronted 2 semicircular translucent response keys separated by a translucent vertical bar. The 2 keys and the bar were independently illuminated from behind. If the bar was lighted, pecking on the darker of the 2 keys caused food to be presented to the bird, and if the bar was dark, pecks on the brighter key brought food. Not all correct pecks were so reinforced. Automatic apparatus scheduled food reinforcements randomly in time. The stimulus array changed frequently in such a way that the left and right keys were correct equally often. Birds were trained in this situation for about 11 hours before drugs were first administered.

Before an experimental session, the pigeon was deprived of food for from 24 to 30 hours, and was deprived of water for about 1 hour. A "warm-up" run of 18 minutes in the subject chamber preceded the oral administration of water or the drug solution. Ten cc. of solution/kg. of body weight were administered. The bird then was replaced in the subject chamber, where it remained for the next 5 hours. Every 18 minutes during this time, the apparatus totaled and recorded the bird's total correct and incorrect responses.

The 5 pigeons served through 2 replications of an experiment involving 5 drugs: chlorpromazine hydrochloride (10 mg./kg.), LSD-25 (200 μg./kg.), meperidine hydrochloride (30 mg./kg.), caffeine (10 mg./kg.), and pentobarbital sodium (10 mg./kg.). For a given bird, a water session alternated with ach 2 drug sessions, each water session serving as a control for both the preceding and the following drug session. The birds had at least 48 hours' rest

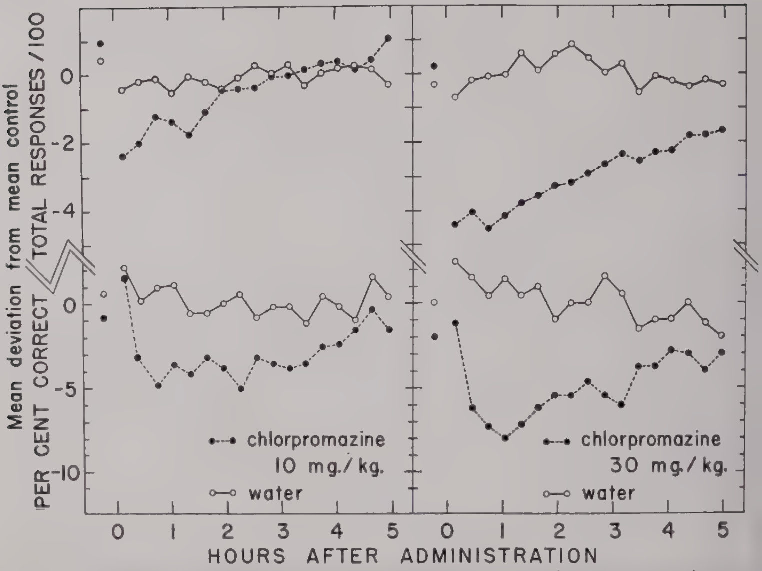

FIGURE 1. The effects of drugs with time following oral administration. Each curve represents the mean of 5 sessions, 1 for each pigeon. Each point represents responding during an 18-minute period.

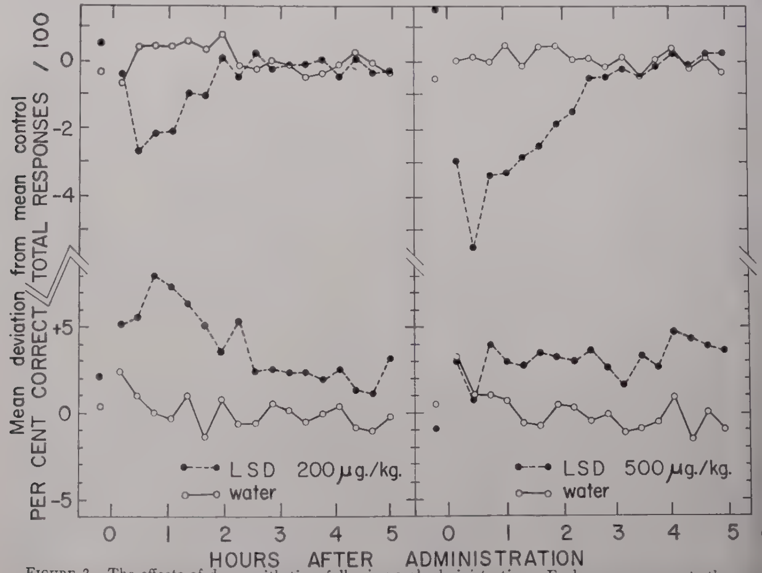

FIGURE 2. The effects of drugs with time following oral administration. Each curve represents the me of 5 sessions, 1 for each pigeon. Each point represents responding during an 18-minute period.

between sessions. A Latin square experimental design was used so that, in a single replication, each bird received each drug once, and every drug appeared once at every position in the order of administration.

At the completion of the 2 replications involving all 5 drugs, the birds had 1 session with a larger dose of LSD (500 µg./kg.) and 1 with a larger dose of chlorpromazine (30 mg./kg.), each preceded by a water control session.

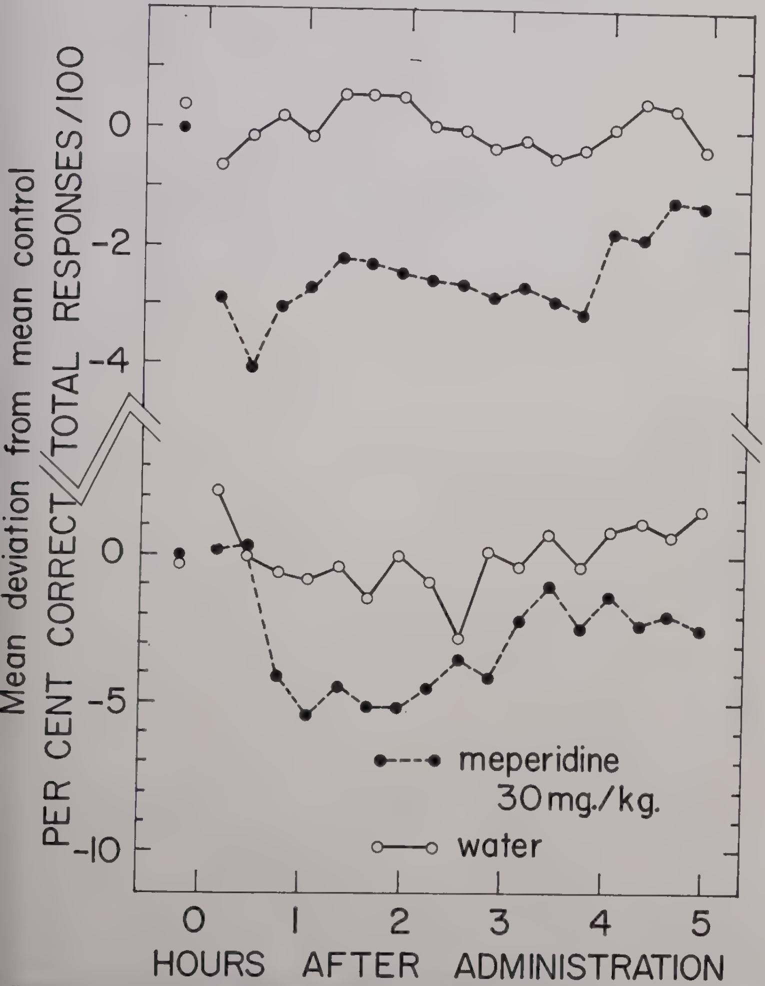

FIGURE 3. The effects of drugs with time following oral administration. Each curve represents the mean 5 sessions, 1 for each pigeon. Each point represents responding during an 18-minute period.

Results

The first experimental replication will not be reported in detail since, at this early stage, the birds performed with relatively high variability. With one exception (meperidine), the average drug effects were all in the same direction as in the second replication, however. Total responses and per cent correct responses from the second replication were averaged for each drug. Averages for the sets of 5 corresponding control sessions were also calculated. Thus, in

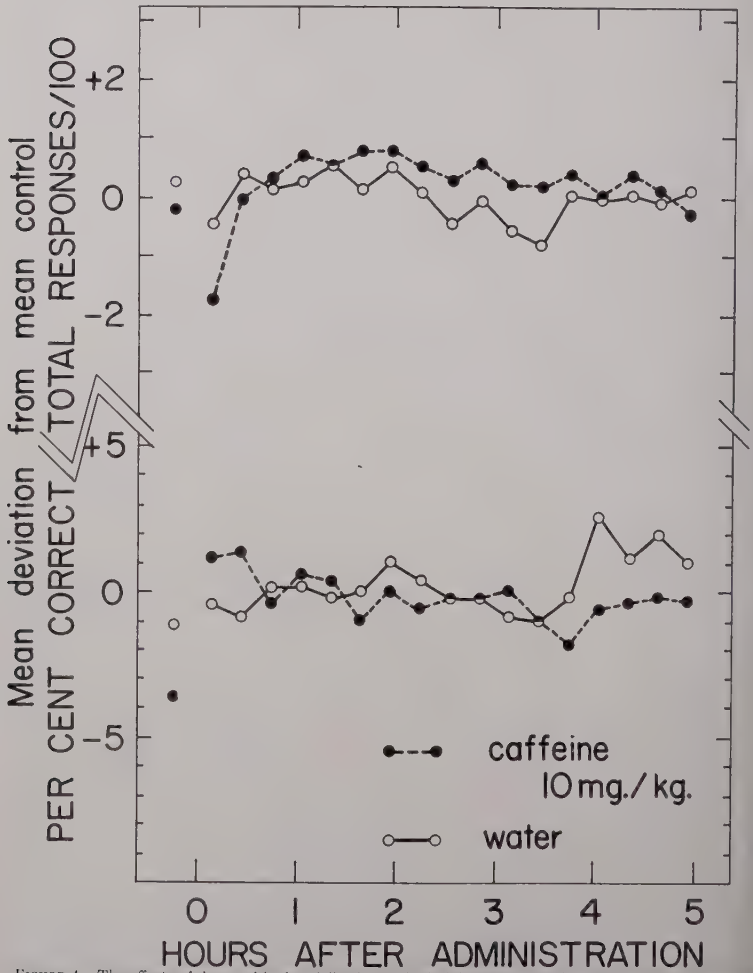

FIGURE 4. The effects of drugs with time following oral administration. Each curve represents the mean of 5 sessions, 1 for each pigeon. Each point represents responding during an 18-minute period.

the figures each curve represents the average of 5 sessions, 1 from each bird. The results with the 2 dosages of chlorpromazine appear in FIGURE 1. The top pairs of curves show total responses to both keys, and the bottom curves show the percentage of correct responses. The 18-minute "warm-up" runs are represented by the lone points to the left. Their usefulness as control points is doubtful, since variability was always relatively high at the beginning of a run. Each point is plotted as a deviation from the mean of all the values obtained during the control sessions.

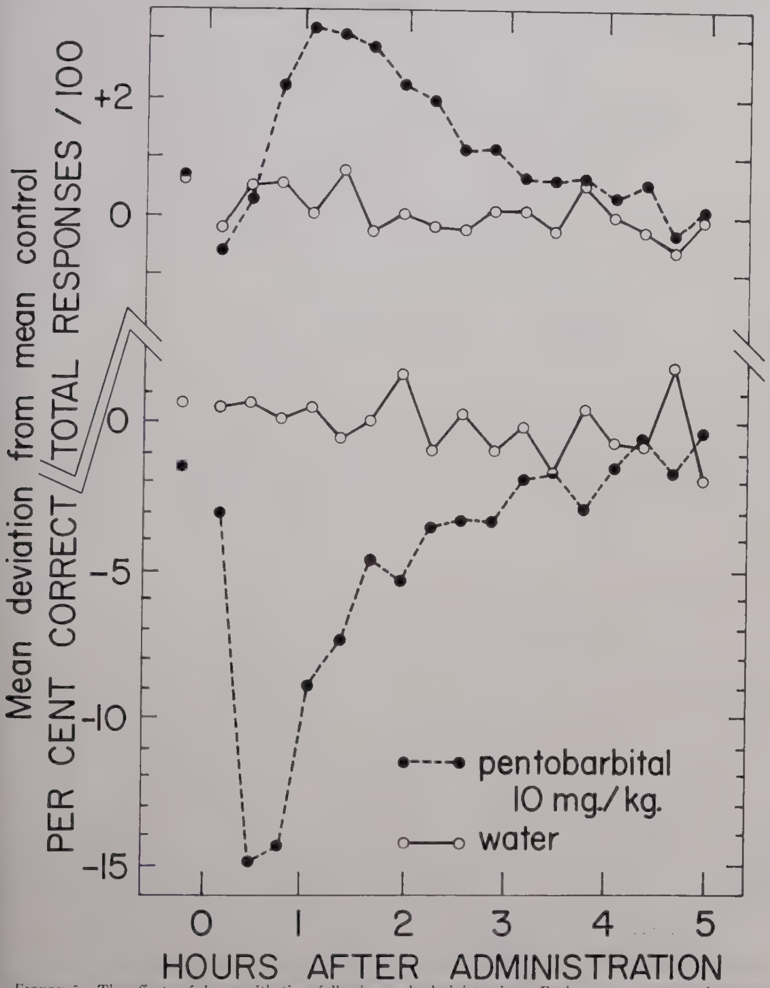

FIGURE 5. The effects of drugs with time following oral administration. Each curve represents the mean of 5 sessions, 1 for each pigeon. Each point represents responding during an 18-minute period.

It appears that at these dosages chlorpromazine decreases both the accuracy of responding and the total response output. The effects increase with the size of the dose, and slowly decrease with time, and there is some indication that the effect on accuracy outlasts the effect on total responses.

The results with LSD appear in FIGURE 2. Again there is an initial decrease in response rate, but this time accuracy is improved considerably by the drug. As with chlorpromazine, there is evidence that the effect on accuracy outlasts the depression of the total response output. Percentage correct was high—over 90 per cent—by the time the high dose of LSD was tested, and its full effects may have been obscured because there was little room for improvement.

In FIGURES 3, 4, and 5 are the results with meperidine, caffeine, and pentobarbital. The heavy dose of meperidine had effects roughly similar to those of chlorpromazine, reducing both accuracy and total response output for a prolonged period. Caffeine at this dosage had no apparent effect on accuracy, and there is a slight indication of an increase in total response output. An increase appeared more clearly in the first experimental replication. Pentobarbital had the most striking effect of all the drugs tested. The marked decrease in accuracy and increase in response output were evident in all birds, though both effects disappeared relatively quickly.

TABLE 1 is a summary of the major findings and also gives some indication of variability. Each plus or minus sign indicates the direction of the difference between a control and an experimental mean. These means are averages of data collected during the period between ½ and 2½ hours after the drug was administered. In 3 cases percentage data are missing because the bird did not respond during that period on the "drug" day. It will be seen that the drug effects are generally consistent, and that (not including caffeine) there are 3 distinct patterns of effects: − −, ∓ −, and − +. This table does not bring out sizable differences in the degree to which the drugs affected different birds.

TABLE 1

DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL DATA COLLECTED IN SINGLE SESSIONS BETWEEN ½ AND 2½ HOURS AFTER ADMINISTRATION OF THE DRUG

		Chlorpromazine				LSD				Meperidine 30 mg./kg.		Caffeine 10 mg./kg.		Pentobarbital 10 mg./kg.	
		10 mg./kg.		30 mg./kg.		200 µg./kg.		500 µg./kg.							
		%	Σ	%	Σ	%	Σ	%	Σ	%	Σ	%	Σ	%	Σ
Pigeon......	06	−	−	*	−	+	−	+	−	−	−	−	+	−	+
Pigeon......	11	−	−	−	−	+	−	+	−	−	−	−	+	−	+
Pigeon......	59	−	−	−	−	+	−	*	−	*	−	+	−	−	+
Pigeon......	76	+	−	−	−	−†	−	−‡	−	−	−	+	−	−	+
Pigeon......	99	+	−	−	−	+	+	+	−	−	−	−	+	−	+

* Bird did not respond during this period on "drug" day.
† Mean control was 98 per cent correct.
‡ Mean control was 95 per cent correct.

Discussion

I can only touch upon a few possible implications of these results. From the pharmacological point of view, the opposite effects of LSD and chlorpromazine on discriminative accuracy are of obvious interest, in view of the preceding discussions on the effects of these drugs. The improvement of a visual discrimination under LSD contrasts with the apparent disruptive effects of this drug on the visual systems of several animals. The relative similarity of the effects of meperidine and chlorpromazine brings to mind reports of similarities in the effects of chlorpromazine and the opiates on escape-avoidance tests. The effects produced by pentobarbital correspond well with those found by Dews[1] under similar circumstances. Caffeine has been found[2] to increase the response rate of rats in an operant situation.

The time dimension of these preliminary data affords a partial substitute for dose-response information, but detailed study at a variety of dose levels clearly is called for before we can generalize widely from the results.

Summary

Five drugs (chlorpromazine, LSD, meperidine, caffeine, and pentobarbital) were studied in pigeons with a new operant-type behavioral technique. The method provided measures of response strength (key pecking) and discriminative accuracy over a period of 5 hours following oral administration of the drugs. All the drugs except caffeine yielded clear effects with 5 pigeons, and 3 general patterns of response were distinguishable.

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BEHAVIORAL EFFECTS OF SOME PSYCHOPHARMACOLOGICAL AGENTS

By Leonard Cook and Edwin Weidley
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Behavioral responses developed in rats have been employed in the evaluation of the comparative effects of several central nervous system (CNS) active pharmacological agents. Differentiation has been made between those CNS active materials that have a *specific* effect on a conditioned response (chlorpromazine, reserpine, morphine), and those that have a somewhat *nonspecific* effect on a conditioned response (barbital, pentobarbital, methylparafynol, meprobamate). The interrelationships between reserpine and serotonin in the CNS have been described by Pletscher *et al.*[1] The effect of serotonin itself upon a conditioned response has also been examined in this study. Lysergic acid diethylamide (LSD) has been reported to antagonize certain properties of serotonin (Gaddum,[2] Shore *et al.*,[3] Costa[4]) and reserpine (Shore *et al.*[3]). We have utilized the conditioned response to examine the interactions of lysergic acid diethylamide with reserpine and serotonin, as well as chlorpromazine, morphine, and meprobamate. Mescaline was also studied, in a manner similar to LSD-25, for possible interaction with conditioned-response blocking agents.

Experimental Technique

Apparatus. The experimental technique originally described by Cook *et al.*[5] employs a pole-climbing response in rats. The experimental chamber shown in FIGURE 1 is approximately 1 foot square. The floor is a grid composed of stainless steel rods three sixteenths of an inch in diameter placed approximately one fourth of an inch apart. The parameters of the shock delivered to the grid floor are controlled by a stimulator that varies both the voltage and the amperage. The stimulator delivers 5 pulses/second. In most experiments a shock of 2 mAmp., 400 v. was employed. The safety area provided is a wooden pole (three fourths of an inch in diameter) with a rough surface to provide maximum grasp for the rat. The pole is attached to the top of the chamber, which also serves as a door, and the bottom end of the pole does not contact the grid floor. The chamber is in a soundproof enclosure (FIGURE 2) to prevent the other rats in the laboratory from perceiving frequent unreinforced conditioned stimuli (buzzer). The buzzer is mounted in the testing enclosure. A timer can be switched into the circuit with either the conditioned-stimulus buzzer (CS) or the unconditioned-stimulus shock (US) to provide a means of timing the "cutoff" period or reaction time of either the conditioned response (CR) or the unconditioned response (UR).

Training. A group of approximately 100 rats is trained to provide a colony for pharmacological studies. A rat is placed in the chamber and a series of shocks (5/second) are delivered to the grid floor concurrently with the activation of the buzzer. This continues for 30 seconds, or until the rat climbs to the safety area (pole), at which time the stimuli are immediately terminated. Ini-

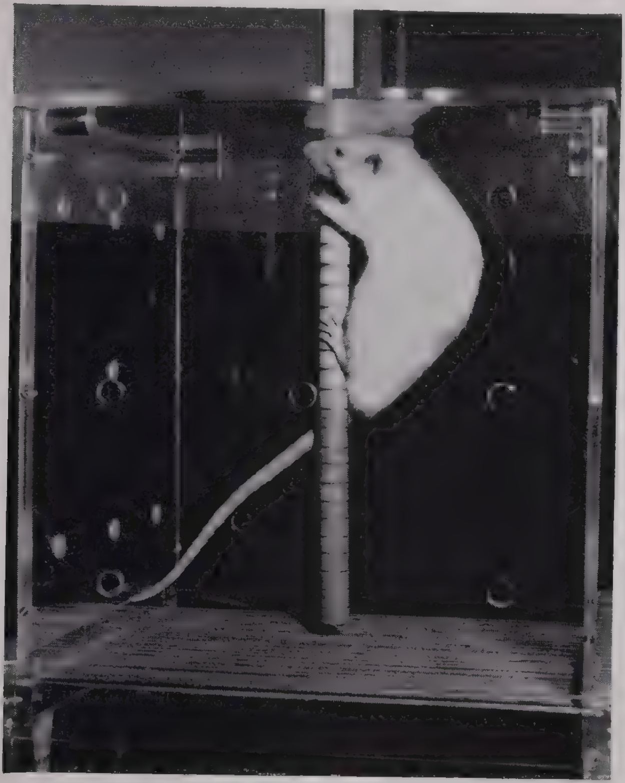


FIGURE 1

tially the rats exhibit random escape movements. After 2 or 3 exposures to this situation, however, they will learn that the pole is the safety area. They are exposed to this situation a few times each day, and after 10 to 15 such exposures they will climb the pole in response to the buzzer only. When this response recurs on a stable basis, a CR is considered to have been developed. Prior to each pharmacological experiment the CR is reinforced a few times with the shock to insure the stability of the CR throughout the day. It has been ound that rats retain the CR over 6 to 8 weeks without reinforcement (associating shock with buzzer). The response developed in rats is termed a *conditioned avoidance-escape response.* There is no time delay between the CS nd the US, both the buzz and the shock are delivered simultaneously during raining. Therefore one cannot truly define this response as a pure conditioned-avoidance response. The CR and the UR are also "holding" responses, since nce the rat responds to either the CS or the US he remains on the pole for an ndefinite period of time and usually has to be replaced onto the grid floor.

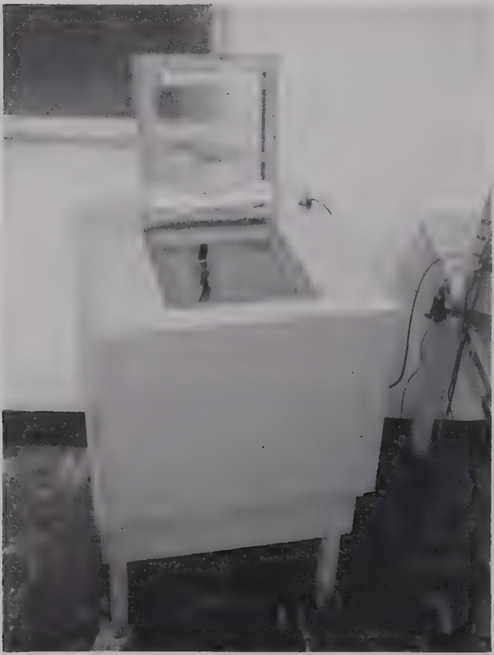


Figure 2

When the extent of training increases, many of the rats develop the secondary conditioned response of jumping onto the pole as soon as they are placed in the testing chamber. The data in this paper do not include effects on this secondary CR, but are concerned with the CR (to the buzzer) and the UR (to shock).

Procedure. In the experiments reported in this paper there were 10 to 15 rats in each treatment group. Control groups received saline injections at equivalent pretreatment times. Data are presented on a quantal basis, that is, the number of rats in a group that were blocked to a specific stimulus (CS or US). A rat was placed in the testing chamber for a period of 30 seconds, without any other stimulus, to allow an accommodation to the situation. If he climbed the pole (secondary CR), he was placed back on the grid floor where he would remain. The CS was delivered for a period of 30 seconds (cutoff) or until the rat climbed the pole, at which time the buzz was terminated. The rat was removed from the pole and replaced on the grid floor, and the US (shock plus buzz) was delivered. The US was terminated when the rat responded (UR) by climbing the pole. The cutoff for the duration of the US was also 30 seconds. Control rats responded within 2 seconds to both the CS and the US, and therefore the cutoff of 30 seconds was beyond the level of statistical significance. The rats were usually tested (first CR and then UR) every 15 to 30 minutes to determine the onset, peak action, and duration of a treatment effect.

There were 3 types of responses generally observed: (1) response within 30 seconds to both the CS and the US, which was seen in the control rats and in those that had received ineffective treatments; (2) failure to respond within 30 seconds to the CS but responding to the US, which indicated a *specific* block of the conditioned response; and (3) failure to respond to both the CS and the US, which indicated a *nonspecific* block of the conditioned response. Failure to respond to the US usually indicated an inability to climb due to an incapacitation of motor function. In certain instances, however, a block of the UR that was not due to such an incapacitation occurred.

In the following experiments the data are presented as the proportion of a group exhibiting a block of the CR and they indicate that the UR was not afected. The exception to this was meprobamate, which also blocked the UR n many of the rats; the degree of *nonspecific* block of the CR is plotted. In hose figures that represent regression lines, each point on the curve represents he maximum effect produced by that dose, regardless of the time it occurred.

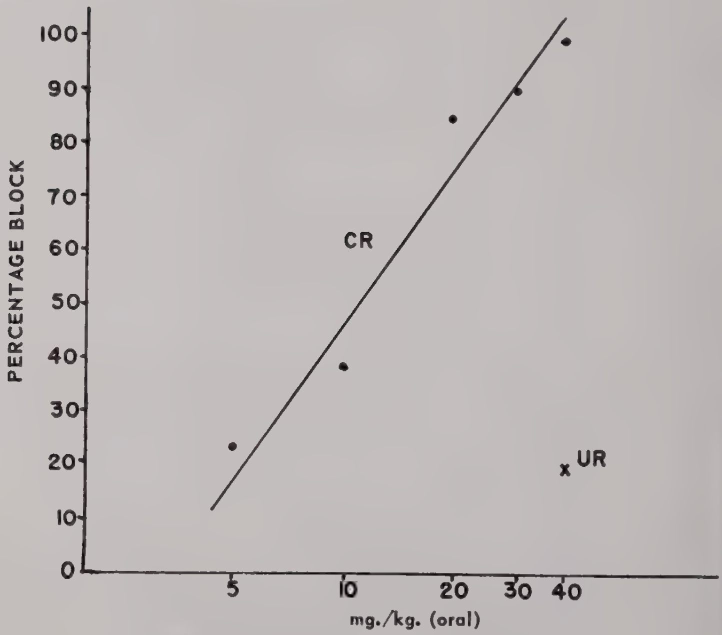
Results

The specific blocking action of the CR produced by chlorpromazine is shown n FIGURE 3. This drug produced minimal effects on the CR after 5 mg./kg., he estimated oral E.D.$_{50}$ for blocking of the CR was 10.5 mg./kg., and it was ound that 40 mg./kg. produced a block of the UR in some of the animals. The aximum effect of chlorpromazine on the CR was produced approximately to 3 hours posttreatment. Some residual effects were seen 24 hours postreatment, and all the animals were responsive to the CS 2 days later.

The effects of barbital, pentobarbital, methylparafynol, and morphine are hown in FIGURE 4. Barbital and methylparafynol produced a nonspecific lock of the CR—the UR (broken line) was affected concurrently with the CR. hese nonspecific effects were produced only after neurotoxic dose levels, as een by the presence of ataxia and semiprostration. Pentobarbital produced a oderate degree of specific block of the CR only after ataxic or semiprostrating ose levels. Morphine sulfate produced a specific block of the CR similar to the ffect of chlorpromazine, and the UR was affected only minimally after 16 g./kg.

Serotonin produced a significant specific block of the CR. The maximum ffect produced after doses of 10 mg./kg. and higher, however, was a 50 per ent block of the CR (TABLE 1). Serotonin also produced a general depression spontaneous motor activity in the animals. The maximal effect of serotonin n the CR occurred between 1 and 2 hours posttreatment. Serotonin produced eath after subcutaneous doses of 150 mg./kg. Analysis of the data showed at there was a significant linear trend of the effect on the CR.

LSD-25 significantly blocked the CR after subcutaneous doses of 1.5 mg./kg. ABLE 2 shows that this dose produced a high degree of specific block of the CR ith a minimal effect on the UR. Higher doses of LSD-25 blocked the UR in creasingly greater proportions of the groups. The block of the UR, how-



IGURE 3. Block of conditioned response—chlorpromazine.

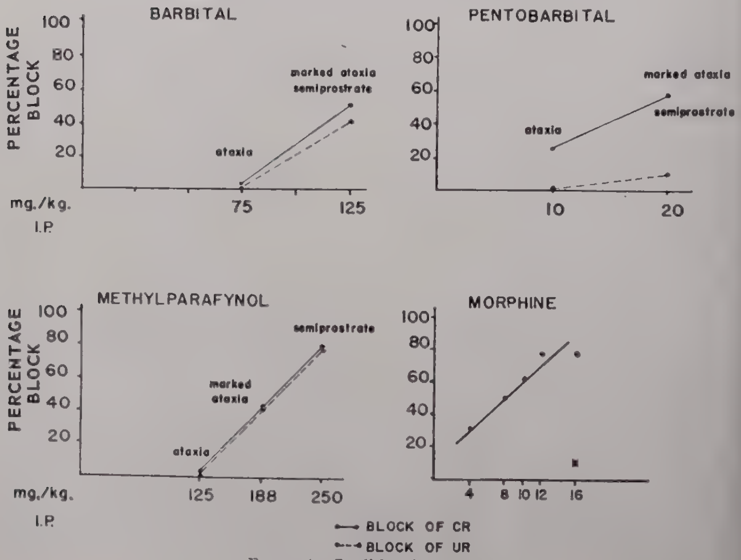


FIGURE 4. Conditioned response.

TABLE 1

EFFECT OF SEROTONIN ON THE CONDITIONED RESPONSE

Mg./kg. subcutaneously	Percentage of group blocked	
	Conditioned response	Unconditioned response
0.5	10	0
2	10	0
5	40	0
10	50	0
20	50	0
40	50	0

ever, did not reflect an incapacitation of pole-climbing ability, as was found by a more detailed examination of the animals. Maximum effects occurred within the first hour posttreatment. Those doses of LSD-25 that produced blocks of the CR also produced hypersensitivity, diarrhea, and frequent urination. Death occurred after subcutaneous doses of 20 mg./kg. In the following experiments, in which LSD-25 was tested as an antagonist to the CR-blocking actions of other agents, subcutaneous doses of 0.1 and 0.5 mg./kg. of LSD-25 were employed that had no measurable effect on the CR.

The CR-blocking action of serotonin was slightly antagonized by 0.1 mg./kg. and significantly antagonized by 0.5 mg./kg. of LSD-25 (FIGURE 5). The maximum degree of antagonism occurred 1 hour after the simultaneous administration of these 2 agents. The general depression produced by serotonin n the rats was also antagonized to an extent by the LSD-25.

LSD-25 was tested for antagonistic properties against orally administered eserpine. It has been found in other experiments that, in rats, relatively arge doses of orally administered reserpine are necessary for effects on this CR. Reserpine is similar to chlorpromazine in that it produces a specific block f the CR; however, the threshold to shock is lowered after the administration f reserpine. Rats were treated orally with reserpine (25 mg./kg.) twice, with 17-hour interval between treatments. LSD-25 was administered subcu- aneously 2 hours after the second reserpine treatment. LSD-25, in doses of

TABLE 2

EFFECT OF LSD-25

Mg./kg. subcutaneously	Percentage of group blocked	
	Conditioned response	Unconditioned response
0.01	0	0
0.1	0	0
0.5	0	0
1.0	0	0
1.5	80	10
2.0	70	30
3.0	70	50
5.0	100	86

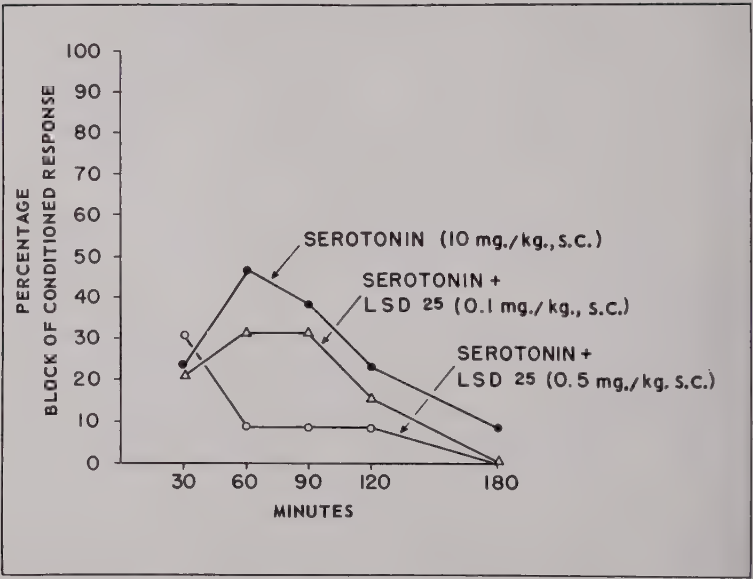


FIGURE 5. Antagonism of serotonin by LSD-25 (conditioned response).

0.1 to 0.5 mg./kg., significantly antagonized the specific blocking action of reserpine on the CR. Maximum antagonism occurred between 1 and 1½ hours after the LSD-25 administration (FIGURE 6). The degree of antagonism diminished after 2 hours, and the level of CR-blocking action exhibited by the combination-treatment groups appeared to approach that of the reserpine control group.

The specific blocking action of the CR produced by chlorpromazine was also significantly antagonized by both 0.1 and 0.5 mg./kg. of LSD-25. The maximum degree of antagonism occurred approximately 90 minutes after the subcutaneous administration of LSD-25 (FIGURE 7) and, as in the case of reserpine the degree of antagonism diminished 2 hours after the LSD-25 treatment.

The action of meprobamate on the CR is qualitatively different from tha produced by chlorpromazine or reserpine. Meprobamate blocks the CR i rats only in neurotoxic dose levels (ataxia, semiprostration), which also block the UR in a large proportion of the group. The action of meprobamate i this procedure was found to be of a nonspecific nature, that is, the animals wer unable to perform the pole-climbing response (CR or UR) mainly due to a incapacitation of motor function. In many instances the animals would at-tempt to respond (climb the pole), but would not be capable of doing so. Th data in FIGURE 8 are presented as the *nonspecific* effect of meprobamate on th CR. LSD-25 was tested in combination with meprobamate to determine if thi type of nonspecific block of the CR could be antagonized. LDS-25 was in-jected subcutaneously one half hour after the oral administration of mepr

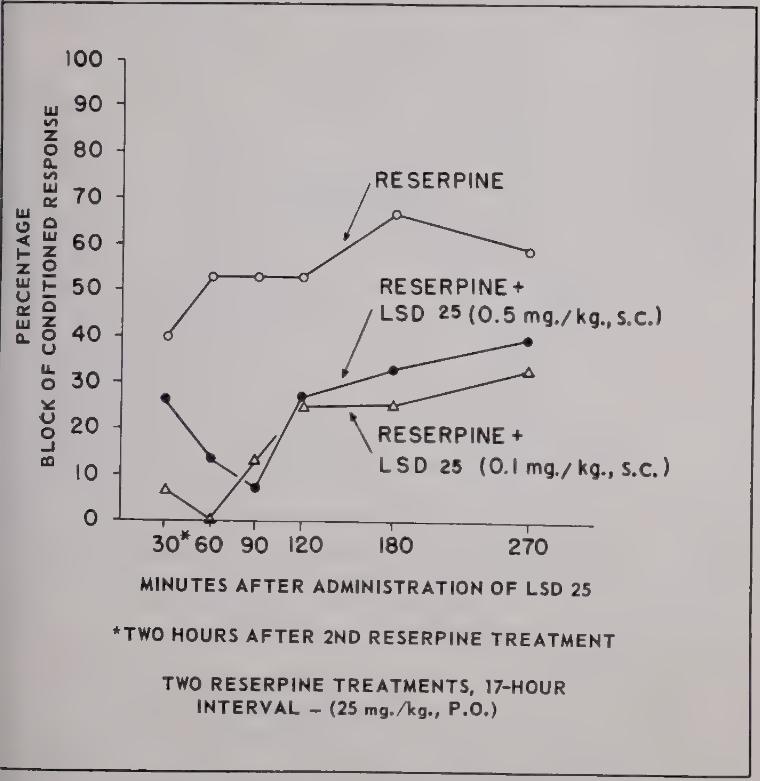


FIGURE 6. Antagonism of reserpine by LSD-25 (conditioned response).

mate, and it was found that LSD-25 (0.1 mg./kg.) significantly antagonized e nonspecific action of meprobamate. The antagonistic action of this dose LSD-25 appeared to diminish after approximately 3 hours. It was noted at 0.5 mg./kg. of LSD-25 failed to antagonize the action of meprobamate on e CR.

It was shown in FIGURE 4 that morphine produced a specific block of the CR. SD-25, in doses of 0.1 or 0.5 mg./kg., which were effective in antagonizing the R-blocking actions of other agents, failed to antagonize the specific blocking tion of CR produced by morphine. FIGURE 9 shows the effect of LSD-25 ministered simultaneously with morphine (12 mg./kg.). It has been shown other experiments, however, that these doses of LSD-25 also failed to tagonize the effects on the CR produced by morphine (4 or 8 mg./kg.) and at LSD-25 (0.01 or 0.05 mg./kg.) did not antagonize the effect of morphine the CR. The data in FIGURE 9 suggest that these doses of LSD-25 may tend enhance slightly the action of morphine on the CR.

The data in FIGURE 10 show that the action of morphine on the CR *is* an-

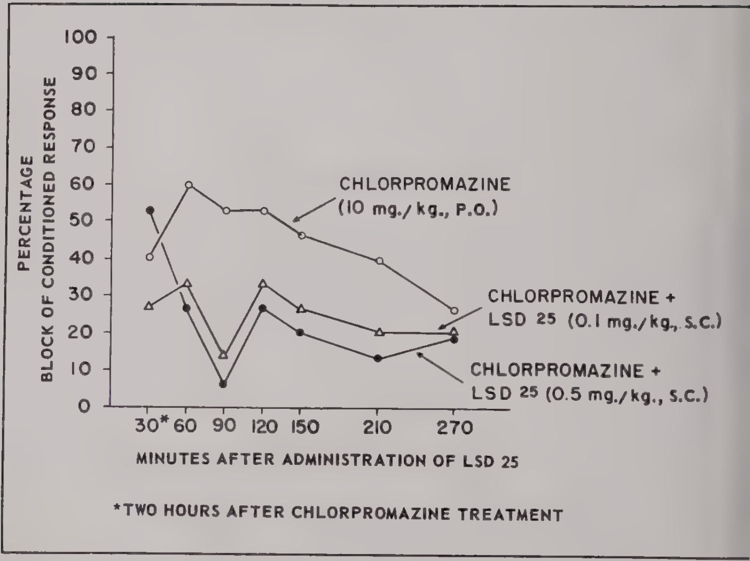


FIGURE 7. Antagonism of chlorpromazine by LSD-25 (conditioned response).

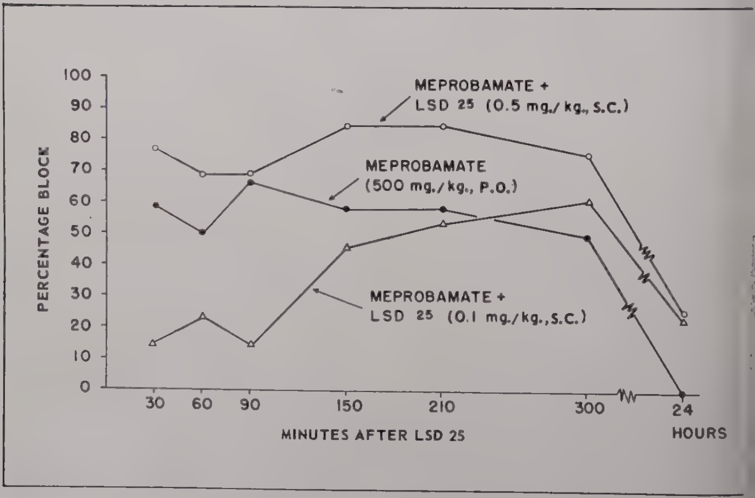


FIGURE 8. Effect of meprobamate and LSD-25.

tagonized by Nalline (N-allyl normorphine). Morphine was administer simultaneously with Nalline (intraperitoneally) and, as shown, Nalline si nificantly antagonized the specific blocking action of morphine on the C Nalline alone, over a wide range of doses, failed significantly to alter the C

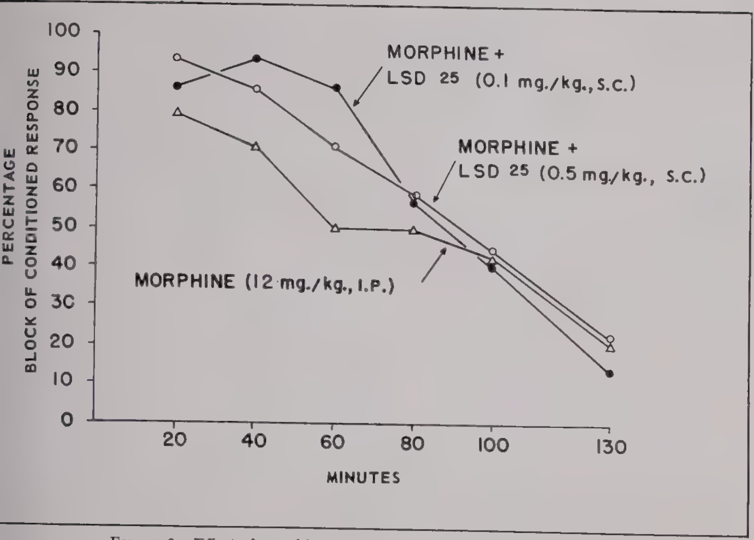


FIGURE 9. Effect of morphine and LSD-25 on the conditioned response.

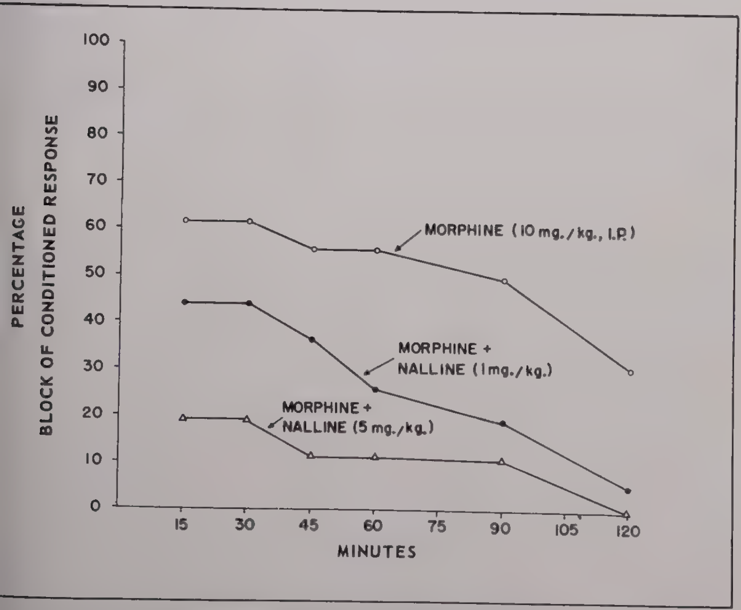


FIGURE 10. Effect of a combination of Nalline and morphine SO_4 on conditioned response in rats.

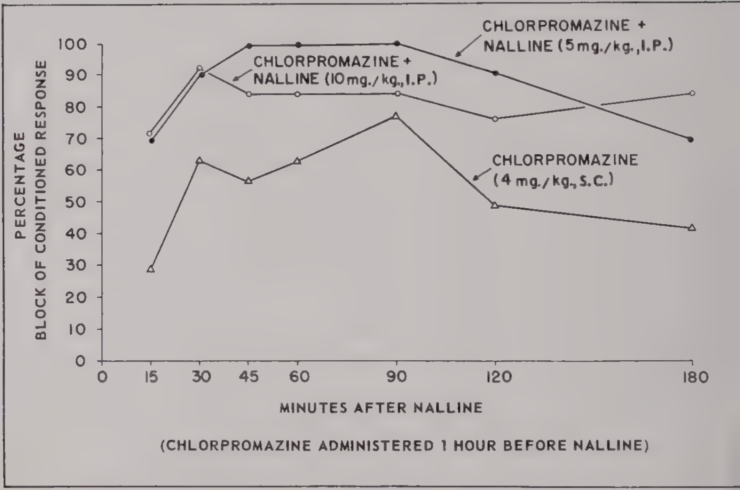


FIGURE 11. Effect of a combination of chlorpromazine and Nalline on conditioned response in rats.

It was also found that Nalline (5 or 10 mg./kg.) failed to antagonize the C blocking action of chlorpromazine (FIGURE 11), and a slight enhancement the CR-blocking action appeared to occur.

Mescaline was found to differ from LSD-25 in that mescaline failed to blo the CR. Mescaline in oral doses ranging from 10 to 100 mg./kg. failed antagonize the CR-blocking actions of chlorpromazine, reserpine, serotonin, morphine. In most instances a slight tendency toward enhancement of t CR-blocking action of these agents was indicated.

Discussion

The technique as described here was designed in an attempt to evaluate t CNS active pharmacological agents for specific effects on a conditioned respo developed in rats. Behavioral responses can be altered by many types of ph macological agents in a nonspecific manner, although relatively few types treatments possess the ability to alter selectively the CR utilized in this stu The UR has been employed as a measure of the animals' ability to perform pole-climbing task. In most instances the UR was an excellent means of eva ating the state of motor function. In a small number of instances, howev the UR was affected by treatments that did not produce an appreciable deg of incapacitation of the motor function. It is essential, therefore, as in techniques involving animal behavior, to observe the animals carefully for gross effects of the treatments employed. Any behavioral response one ploys, usually simple and restricted with respect to the experimental criteri based upon complex mechanisms, and alterations of the response can be acc plished by many different types of mechanisms, as well as in the presence ariety of other pharmacological effects. It is therefore quite apparent that imilar types of alteration of any restricted criteria of behavior do not neces- arily indicate similar mechanisms of action. The variety of pharmacological gents that alter the conditioned response employed in these studies best illus- rates these points.

The CR was specifically blocked by chlorpromazine, reserpine, and morphine. he regression line for the effect of chlorpromazine is shown in FIGURE 3. It as found that chlorpromazine blocked the CR specifically without blocking he UR until much higher doses were administered. Reserpine blocked the R in a similar manner, although it was found necessary to administer large oses for 2 daily treatments in order to produce an effect on the CR with orally dministered reserpine. A marked increase in sensitivity to the US occurred in eserpine-treated rats.

Morphine specifically blocked the CR in a manner similar to both chlorproma- ine and reserpine, and the response to the US was not altered until large doses f morphine were administered. It was interesting to note that serotonin roduced a specific block of the CR, although an effect in 50 per cent of the roups was the maximum degree of block observed. Serotonin depressed spon- neous locomotor activity in rats as well as in mice. It was found that erotonin produced only depression at all dose levels up to lethal doses, and imulant properties were not observed. A specific block of the CR was also roduced by LSD-25, although the UR was blocked in a certain proportion of e groups tested (TABLE 2). The fact that the CR can be blocked in the pres- nce of a variety of other pharmacological effects is best illustrated by the effects chlorpromazine (animals indifferent to most external stimuli) and LSD-25 nimals hypersensitive).

Barbital and methylparafynol produced a *nonspecific* block of the CR, affect- g the UR concurrently due to an incapacitation of the motor function. A oderate degree of specific CR-blocking action was produced by pentobarbital, though the effective doses also produced other neurotoxic effects. Mepro- mate blocked the CR only in neurotoxic dose levels (ataxia), and it affected e UR in many of the animals.

Two types of actions of LSD-25 have been demonstrated. High doses block e CR, and lower doses that produce no measurable effect on the CR antagonize e CR-blocking actions of several other drugs. The antagonistic effect of SD-25 against the CR-blocking action of serotonin shows that on a gross havioral level there is some agreement with respect to the interaction of these ents and with the findings of Gaddum,[2] Shore *et al.*,[3] and Costa,[4] who showed tagonism by *in vitro* and *in vivo* procedures. LSD-25 antagonism of reserpine ects (prolongation of hexobarbital hypnosis) has been reported by Shore *et* [3] The CR-blocking actions of reserpine have also been found to be antago- zed by LSD-25.

LSD-25 also antagonized the effect of chlorpromazine. The antagonistic tion of LSD-25 against the effects of both chlorpromazine and reserpine owed a maximum effect about 90 minutes after its administration. The ration of this maximum effect was relatively short, as shown in FIGURES 6 and 7. Residual antagonistic effects lasted for a few hours, although in ea instance the actions of the combination treatments appeared to approach tho of the control treatments. The nonspecific action produced by meprobama was antagonized only by 0.1 mg./kg. doses and not by 0.5 mg./kg. doses LSD-25. The antagonistic action of LSD-25 diminished after approximate 3 hours, at which time the group that had received the combination treatme exhibited a degree of response equivalent to that of the control group. T failure of LSD-25 to antagonize the action of morphine is most interesting a leads to a number of possible speculations. It may indicate that the mech nisms responsible for the CR-blocking action of morphine are different fro those of chlorpromazine and reserpine, even though the restricted criteria behavioral response were altered in a similar fashion, and that LSD-25 a tagonism offers a means of differentiating between certain types of speci CR-blocking actions. The possibility of a differential mechanism of acti on the CR of morphine as compared with chlorpromazine is further enhanc by the finding that Nalline antagonized the CR-blocking action of morphin but failed to antagonize the effect of chlorpromazine. These data provo much speculation on the mechanisms of the action of analgesia.

Mescaline was found to differ from LSD-25 with respect to the properti evaluated in these studies. Mescaline failed to produce any significant blo of the CR and was not found to antagonize the CR-blocking actions of chlo promazine, reserpine, serotonin, or morphine. In most instances, however, slight degree of enhancement of the CR-blocking actions of these compoun was produced by mescaline.

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HE INHIBITION OF THE CONDITIONED RESPONSE AND THE COUNTERACTION OF SCHIZOPHRENIA BY MUSCARINIC STIMULATION OF THE BRAIN*

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Numerous investigators have studied the inhibition by reserpine and chlor-
romazine of various types of conditioned responses (CR) in animals.[1, 2, 3, 4]
his inhibition occurs at a dosage that is a small fraction of the lethal dose of
ılorpromazine. This inhibitory effect is evidenced by reserpine, 11-des-
ethoxyreserpine, and rescinnamine, all of which are active tranquilizing drugs.
ther chlorpromazinelike compounds are either inactive or produce this inhibi-
on of the CR at a dosage level that impairs the physical activity of the animal.
zacyclonol (Frenquel) and meprobamate are completely inactive.[4] 5-Hy-
oxytryptamine (5 HT) in doses of 50 mg./kg. produces marked symptoms in
e rat, but it does not inhibit the CR.[4] *d*-Lysergic acid diethylamide (LSD)
doses of 1 to 2 mg./kg. inhibits the CR of the rat, but the animals appear so
nfused that the specificity of any similarity to the chlorpromazine effect is
estioned.[4]

Since many of the pharmacological data in animals and clinical studies in
tients show that the active alkaloids of *Rauwolfia* and, to a lesser extent,
lorpromazine, have a persistent acetylcholinelike effect, we chose to investi-
te the effect on the CR of various muscariniclike drugs that will pass the
ood-brain barrier freely. The tertiary amines arecoline, pilocarpine, and
erine were therefore studied, and only a few experiments were made with
ethacholine and acetylcholine—both quaternary amines.

Atropine, a tertiary amine, passes the blood-brain barrier freely. Atropine
ould protect the animals from the peripheral effects of arecoline, eserine, and
locarpine, but it cannot be used since it would also protect the brain from
e muscarinic effects of these drugs. Methyl atropine nitrate (MeA, Eumy-
ine) is a quaternary nitrogen analogue of atropine that probably does not
ss the blood-brain barrier. MeA was therefore used to protect the rats
m the peripheral effects of the parasympathetic stimulants while the brain
s unprotected, and we could therefore observe the effect of cerebral musca-
ic stimulation in animals that were not salivating, defecating, or anoxic
m pulmonary edema.

Methods

The conditioning box consists of the usual 15-inch × 15-inch square box with
ass bars spaced at 1-cm. intervals for a floor. The rats can escape the floor
the sound of a buzzer by jumping up a center pole (CR). After the buzzer

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The trials on the schizophrenic patients were carried out under the supervision of William J. Gallagher, Physi-
-in-Charge of the Schizophrenic Research Ward at Manteno State Hospital, Manteno, Ill., and were sup-
ed by a grant from the Mental Health Fund of the Illinois Department of Public Welfare, Springfield, Ill.

has sounded for 3.0 seconds an alternating current of 200 μAmp. is passe through the floor grid, at which time the rat may jump up the center pole (unconditioned response—UR). When given drugs that inhibit the CR and UR the rats may show no response to the stimulus. This is termed "take shock (TS). The training of the rats is started when they weigh only 50 to 70 gram With the use of these young animals, we do not encounter any rats that canno be conditioned, nor do we encounter any neurotic behavior before body weight of 150 to 250 gm. have been attained. In young animals the learning proces is rapid. The rats can be well-trained within 7 to 10 days, with an averag time in the cage of 10 minutes per day. Care must be taken to avoid larg doses of reserpine (above 0.75 mg./kg.), since these animals are erratic in thei conditioned responses for at least 1 month after a single dose. Arecoline eserine, and pilocarpine do not produce this chronic effect, so that we wer able to use the individual rat as his own control in all instances. Since a doses were parenteral, we did not restrict the rats' food on the night befor the day of the experiment. Rats were trained to a performance level of 1 out of 10 positive conditioned responses prior to their use in an experiment This performance was retested prior to each drug experiment. A defecatin rat will not respond to the buzzer, and therefore a mechanical programmin device was not used. If a test rat was defecating at the time of the buzze the animal was not shocked, but tested with the buzzer signal 10 seconds late Properly trained rats exhibit no fear of the conditioning cage. This is manifested by lack of urination and defecation, and the animals roam about the cag in a normal manner.

Results

Arecoline (2 mg./kg. administered subcutaneously) in rats protected wit MeA (20 mg./kg. administered intraperitoneally) produces fine tremors of th

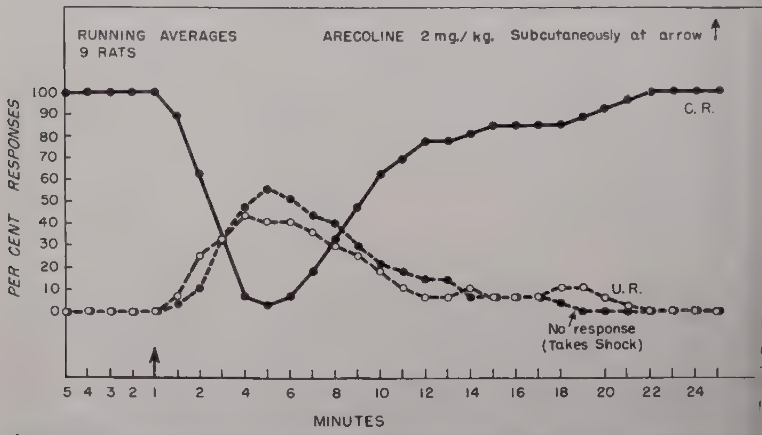

FIGURE 1. The effect of arecoline alone on the conditioned response (CR) of the rat. The rats had sympto of muscarinic stimulation during inhibition of the CR.

ead, tail, and extremities. The rats have no salivation, tearing, or diarrhea. The inhibition of the CR, however, begins before this central nervous system CNS) stimulant effect and lasts several minutes after the rat appears normal FIGURES 1, 2, and 3). Atropine prevents this effect on the CNS. The maxinal effect on the CR appears at 5 minutes, and the animals are back to normal rained behavior in 20 minutes. Intravenous doses of arecoline (75 mg./kg.) n the mouse protected by MeA (25 mg./kg. intraperitoneally) produce marked xcitement and tremors, but no convulsions and no deaths. Eight out of 10 ontrol mice without MeA died at a dose of arecoline (50 mg./kg.).

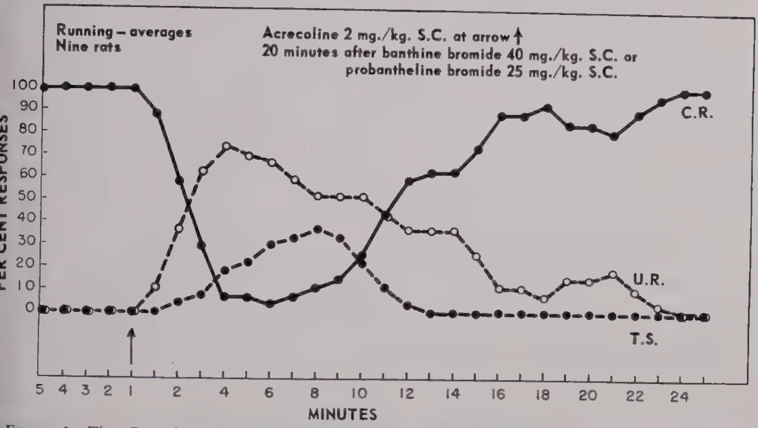

FIGURE 2. The effect of arecoline on the conditioned response (CR) of the rat protected by probantheline omide. The animals showed slight tremor at the height of the action of the drug.

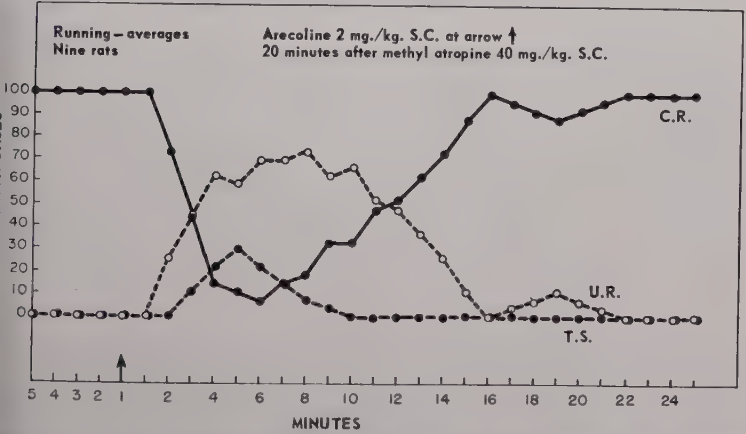

FIGURE 3. The effect of arecoline on the conditioned response (CR) of rats protected by methyl atropine. e rats had no muscarinic symptoms but showed a fine tremor at the 5- to 7-minute interval.

Pilocarpine (8 to 10 mg./kg. subcutaneously) in rats protected with MeA (2
mg./kg. intraperitoneally) produces no tremor. The animals appear phys
cally normal, but they do not retain their conditioned performance. The max
mal inhibition of the CR occurs at 20 minutes, and the rats are back to norm
trained performance in 90 minutes (FIGURE 4). They have no signs of para
sympathetic stimulation. Atropine prevents this effect on the CNS almo
completely.

Eserine in subcutaneous dosage of 0.25 mg./kg., either alone or in Me
protected rats, produces a maximal inhibition of the CR at the 20- to 40-minu
test period. The animals protected by MeA (10 mg./kg.) show slight trem
from the eserine. The rats return to their previous CR performance in
minutes (FIGURE 5). Atropine prevents completely the inhibition of the C
produced by eserine.

In the unprotected rat a dose of 0.5 mg./kg. of methacholine will produ
chromodachorrhea, salivation, and defecation. In the rat protected wi
MeA (10 mg./kg. intraperitoneally), doses of methacholine as high as
mg./kg. produce no symptoms of parasympathetic stimulation, no signs
CNS stimulation, and no inhibition of the CR!

After the failure of methacholine to produce an inhibition of the CR
determined whether acetylcholine in the neostigminized (0.05 to 0.1 mg./k
and methyl-atropinized (5 to 20 mg./kg.) rat would have any effect on the C
In the methyl-atropinized and neostigminized rats, doses of 5 to 20 mg./kg.
acetylcholine produced marked peripheral symptoms of nicotinic action, wi
muscle fasciculations plus some salivation and pulmonary edema. Acety

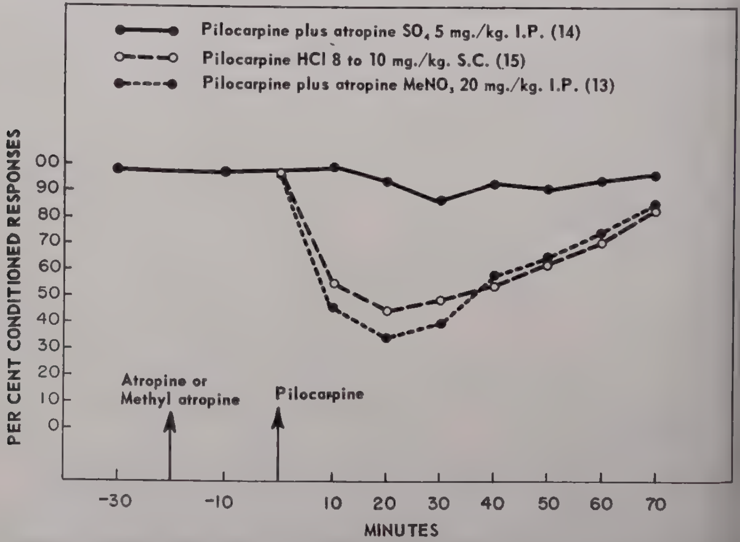

FIGURE 4. The effect of pilocarpine on the conditioned response (CR) of the rat. The rats had no symptc
when proteced by methyl atropine.

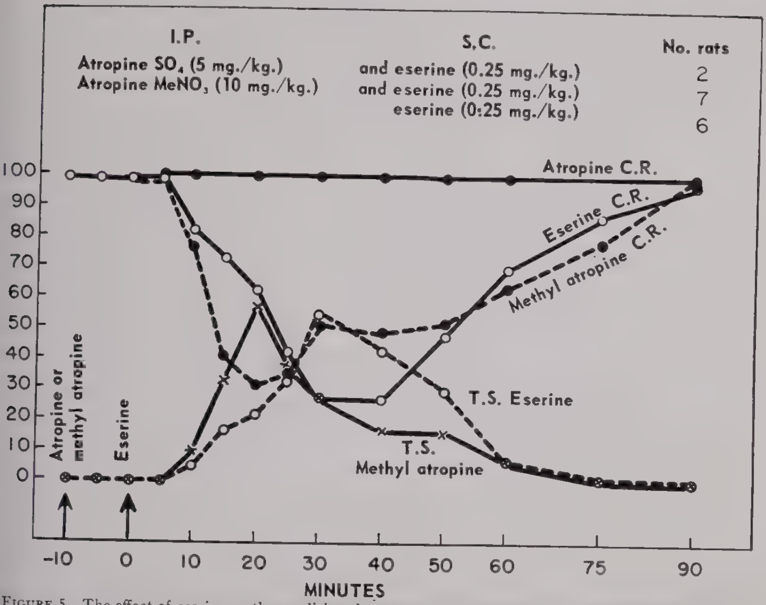

FIGURE 5. The effect of eserine on the conditioned response (CR) of the rat. The rats showed fine tremor he height of the action of the drug.

oline had no definite effect on the CR in spite of these incapacitating symp- ms.

Theoretically, atropine should modify the known inhibition of the CR that produced by small doses of chlorpromazine or reserpine. This antagonism, wever, must depend on the selective affinity of the 2 competing blocking ugs for the receptors in the CNS. We know that the pharmacological effect both chlorpromazine and reserpine lasts longer than does the effect of ropine. This could mean a greater affinity for receptors. We were thus t too disappointed when we could not demonstrate any statistically signifi- nt effect of atropine (10 mg./kg. intraperitoneally) on the inhibition of the produced by reserpine (0.75 mg./kg. subcutaneously). Similarly, we ıld not demonstrate any statistically significant effect of intraperitoneal opine (10 mg./kg.) on the inhibition of the CR produced by a 4 mg./kg. se of chlorpromazine given subcutaneously.

Trials in Schizophrenic Patients

ur first observations were made with eserine in schizophrenic patients pro- ted with probantheline bromide (Pro-Banthine) in subcutaneous doses of mg. This dose resulted in acceleration of the heart rate up to as much as per minute. In subsequent use of probantheline, we used a dose of 15 mg. t uniformly produced a heart rate of 120 to 140 per minute. Five to 10 minutes after the probantheline, eserine was given cautiously in increasin doses until a total dose of 5 mg. was attained. The 2 patients in which th was tried became more talkative and showed greater insight and sociabilit Sweating, salivation, or diarrhea did not occur.

We next tried increasing doses of arecoline cautiously in 6 patients protecte with subcutaneous doses of 15 mg. of probantheline. With a subcutaneou dose of 10 mg. of arecoline, fine tremors, hyperreflexia, yawning, sighing, resp ration, and exploratory movements of the hands about the face and head o curred. Two patients had spells of dry sobbing, 1 patient attempted to escap from the hospital by way of a window, and 2 patients had lucid intervals tha were more informative than were previous interviews under amobarbital. Th onset of drug effects from both arecoline and probantheline is extremely rapi and therefore the time interval between the protective dose of probanthelin and the excitatory dose of arecoline was reduced gradually. When both drug were given simultaneously, the patients did not show salivation, sweating, o tearing, and more extensive studies were then undertaken with 3 mg. of methy atropine and 20 mg. of arecoline mixed and injected subcutaneously with th same syringe.

In 23 trials of this combination (TABLE 1) we have observed a tachycardia u to 140 per minute, and a rise in both systolic and diastolic blood pressure of 1 to 20 mm. of mercury. No peripheral muscarinic symptoms occurred. Tw patients vomited 1 to 2 hours after being given 40 mg. of arecoline, and patient vomited within 5 minutes after a dose of 20 mg. of arecoline and 3 m of methylatropine. Most patients exhibit a facial flush within 2 minute after the dose, and a few others have facial pallor at the 8- to 15-minute inter val after dosing. Both of these signs we attribute to the effect of methy atropine. The facial flush is commonly seen with atropine, while the delaye pallor may be due to ganglionic blockade by the methyl atropine.

The antischizophrenic action of arecoline begins 1 to 2 minutes after injec tion, reaches a peak in 5 minutes, and disappears completely in 15 to 2 minutes. A few patients who were receiving concomitant therapy wit iproniazid appeared to have a slightly more prolonged effect from the arecolin Concomitant therapy with moderate doses of reserpine (2 to 4 mg./day) o chlorpromazine (300 mg./day) does not modify or exaggerate either the pharma cological or behavioral effects.

While several of our completely mute patients did not talk while under th influence of arecoline, they did show an increased interest in their surrounding changed body posture, and felt their faces with groping movements of the hands. One of the patients (G. E.) who attempted escape after arecoline i variably attempts escape when his psychiatric state has been improved b convulsive therapy. The basic signs of improved emotional outlet such laughing, sobbing, sighing, and yawning after arecoline are noteworthy in th they are absent uniformly in the untreated institutionalized schizophren patient.

We have tried pilocarpine (after methyl atropine) in 1 patient who was give 2 subcutaneous doses of 10 mg. without change in his behavior. Since we a

TABLE 1

RIALS OF ARECOLINE IN SCHIZOPHRENIC PATIENTS PROTECTED BY QUATERNARY ATROPINES

Schizophrenic patient	Sex	Drugs		Reaction
		MeA.	Arecoline	
L. S.	F	15*	2, 4, 10 mg.	More talkative, apprehensive, escape attempt, quieted, sobbing
G. E.	M	15*	15, 15	More talkative, apprehensive, escape attempt
G. W.	M	15*	5, 10	Sobbing (as with amytal)
R. B.	M	15*	15, 10, 10	Excellent lucid interval (better than amytal)
R. H.	M	15*	10, 10	Good lucid interval
R. K.	M	15*	10, 10	Good lucid interval
A. K.	M	3	20, 20	More attentive, still mute
E. R.	F	3	20, 20	Excellent lucid interval
L. W.	M	3	20, 20	Good lucid interval (better than amytal)
S. L.	M	3	20	Good lucid interval, tremors
C. H.	M	3	20, 20	Good lucid interval (vomited 90 min.)
C. P.	M	3	20	Restless, searching movements, still mute
R. H.	M	3	20, 20	Good lucid interval (vomited 60 min.)
R. C.	M	3	20	Excellent lucid interval, informative
J. S.	M	3	20	Yawning, sighing (no lucid interval)
J. K.	M	3	20	Belligerent—good lucid interval
B. B.	M	3	20	Vomited—10 min. Fair lucid interval
R. C.	M	3	20	Excellent lucid interval
J. W.	M	3	20	Restless, abortive attempts at speech, still mute, questionable nausea
S. C.	M	3	20	Continuous sobbing, apprehensive, escaped from room
M. B.	M	2	15	Laughter, excellent lucid interval
W. J.	M	2	15	Good lucid interval, talked about himself clearly in third person, slight sweating
R. B.	M	3	Pilocarpine 10, 10, Arec. 10	Laughter, good lucid interval

* Probantheline bromide.

Most patients showed yawning, sighing, respiration, hyper-reflexia, searching and groping movements of nds about face. Facial flush occurred at 2 to 5 min. followed by pallor and occasional tremor at 8 to 10 n.

ot as pharmacologically confident that pilocarpine has only muscarinic stimu-
nt action, and since the usually recommended dose of pilocarpine is 5 mg., we
opped at this dose of 20 mg. in order to pioneer, on another day, with larger
ses of pilocarpine. Since 3 out of 21 patients vomited with 20+ mg. of
ecoline plus 3 mg. of methyl atropine, we recommend for purposes of psychiat-
interviews the lower dose of 15 mg. arecoline plus 3 mg. methyl atropine
1 ml. of sterile saline injected subcutaneously just above the elbow so that a
nous tourniquet can be applied to stop absorption if any untoward reaction
curs. Of the 3 possible tertiary amines with parasympathetic stimulant
tion, arecoline appears ideal for interview purposes in that the effect of a
bcutaneous injection begins in 2 minutes and lasts from 5 to 10 minutes.
ur animal data indicate that pilocarpine and eserine should be slower in onset
d have longer durations of action. With wider experience, arecoline plus
ethyl atropine may prove useful in the preliminary screening of patients for
serpine therapy.

Arecoline plus methyl atropine is unique among drugs recommended for

psychiatric interview of the schizophrenic patient in that the lucid interva
when it occurs, is accompanied by stimulation of the brain by a recogniz
pharmacological stimulant action. Lucid intervals after carbon dioxide
amobarbital occur with overt depression of the CNS, and the many possib
mechanisms of drug action are obviously much more difficult to analyze.

Discussion

Funderburk and Case[5] have found that eserine inhibits the CR in the ca
The cats, however, had marked peripheral parasympathetic symptoms th
may have distracted their attention from the buzzer signal. The findings
Gellhorn[6] are also suggestive. Gellhorn found that treatment with atropi
or thyroxin tended to restore the conditioned response when it had been i
hibited by insulin or by electrically induced convulsions. This improvemer
however, was attributed to "an increase in central sympathetic activity
The studies of Jacobsen[7, 8] in which the conditioned response is facilitated b
synthetic atropinelike compounds is perhaps the pharmacological antithes
of the inhibition by arecoline of the conditioned response.

We have purposely used the term "muscarinic action" to describe the actio
of arecoline, pilocarpine, and eserine on the CNS. While the term "muscarin
stimulation" of the brain is used deliberately in this report, we do not wish
transfer to the brain the classical concept of increase in glandular secretion
smooth-muscle activity that is inherent in the pharmacological action of mu
carine on the parasympathetic nervous system. We do wish to imply that th
is the action on the CNS of an unbalanced acetylcholinelike molecule that h
greater muscarinic than nicotinic effects. Neither of these 2 actions of acet
choline has been defined accurately in terms of brain function. Of the 2 div
sions of known pharmacological action of acetylcholine, one might assun
a priori that the most important action on the CNS is the "nicotinic actior
(the important action in transmission at ganglia and skeletal muscle). Fel
berg[9] points out that acetylcholine applied to the cortex can be counteract
by atropinization, which suggests a muscarinic action of acetylcholine on t
brain. He further points out, however, that some of the nicotinic effects
acetylcholine at ganglia are also blocked by large doses of atropine. The dru
arecoline and pilocarpine are almost devoid of nicotinic action, however, a
they have prominent muscarinic action. Furthermore, muscarinic action
synonymous in our thinking with the action of methyl on carbon and oxyg
atoms of the acetyl end of the acetylcholine molecule. For example, in so
tests the acetylation of choline increases the muscarinic action by a factor
10^5. Atropine blocks the muscarinic action of acetylcholine and, from c
viewpoint, blocks the receptors for the oxygen end of acetylcholine. V
further postulate that the nicotinic end of acetylcholine resides mainly in t
quaternary nitrogen or onium head. This is essentially pressor, and t
pharmacological action is blocked by hexamethonium, decamethonium, a
competitively inhibited by tetraethylammonium. The free onium head its
tetramethylammonium, mimics the nicotinic actions of acetylcholine.
similar fashion, acetaldehyde (in the "Antabuse reaction") and carbon diox

nimic, to some extent, the muscarinic actions of acetylcholine, and the cortical lepressant effects of CO_2 can be prevented by atropine.[10] If one accepts the act that the glandular secretory effect and vasodilation produced by carbon lioxide simulate, in part, the muscarinic action of acetylcholine, then the lucid nterval in schizophrenics[11, 12] that is produced by the inhalation of carbon lioxide is plausibly understandable and in accord with our experimental data. his working hypothesis would then account for the findings of Medinets, line, and Mettier,[13] who report a prolonged lucid interval in schizophrenic atients after the administration of dibenamine by intravenous infusion. owntree, Nevin, and Wilson[14] have shown that the nicotinic stimulation of he brains of schizophrenics that results from daily intramuscular administra-ion of the anticholinesterase agent diisopropyl fluorophosphate increases their egree of schizophrenia, but benefits the mania of manic-depressive insanity.

Three of the physiological actions of the psychotomimetic drug LSD are imilar to those of atropine. These are the facial flush, dilatation of the pupil, nd rise in body temperature. One can add as additional similarities the mild achycardia and hypertension that LSD produces in man. LSD in doses of 1 to mg./kg. inhibits the conditioned response in the rat,[4] but we are not certain hat this is a specific effect or is owing to the confusional state produced by this ose. Theoretically, very small doses of LSD-25 should facilitate conditioning nd block the arecoline inhibitory effect, while atropine should enhance the SD-25 psychosis. Quigley[15] found that 0.6 to 1.2 mg. doses of atropine pro-uced confusion in a majority of normal volunteers who had been injected oncomitantly with 20 units of insulin. This may have been a summation the hypoglycemia and the atropine effect or an interaction of the pharma-ological effects of atropine and epinephrine released by the hypoglycemia. e know that atropine in doses of 6 to 8 mg. will produce delirium in susceptible dividuals.

Kosman and Gerard[16] find that large doses (6 mg./kg.) of epinephrine in-bit the CR in the rat. In our hypothesis, epinephrine is one of the most otent, short-acting, antimuscarinic compounds available. One would expect nall doses of epinephrine to facilitate the CR as does amphetamine, so that is 6-mg./kg. dose must be again a confusional dose. Any derivative or gradation product of epinephrine that is stable and strongly antimuscarinic eoretically should produce hallucinations. Norepinephrine, with the phenyl-ng oxygens or hydroxyls stabilized by methyl groups, might be such a com-ound (this compound shows a striking resemblance on paper to the chemical ructure of mescaline). Adrenochrome, adrenolutin, other degradation oducts of epinephrine, or 5-hydroxytryptamine (5 HT) might be more rongly antimuscarinic for the brain.

One of the known metabolic defects that can produce seizures, mental retarda-on, and insanity is the inability to utilize phenylalanine so that large amounts phenylpyruvic acid (PPA) accumulate in the body and are excreted in the ine. A careful consideration of the formula for PPA shows that this is a olecule designed neatly to act as a blocking molecule for the oxygen or ester d of acetylcholine. Thus, PPA should be antimuscarinic or atropinelike

(FIGURE 6). This hypothesis can be tested by the determination of th atropinelike effect of PPA or its synergistic effect with LSD. Perhaps th amide or methyl ester of PPA should also be tested, since these molecules woul penetrate the CNS more easily than would the free acid.

Since reserpine liberates 5 HT from its bound form in the brain[17] and is ant schizophrenic, one wonders how this may be correlated with the present finc ings. 5 HT alone does not affect the CR in rats,[4] so the free 5 HT can hardl be the corrective neurohumoral factor. The schizophrenic patients probabl lose most of their cerebral 5 HT within a week, but their progress toward sanit requires 2 to 3 months. Perhaps when reserpine decreases the 5 HT in th brain the muscarinic functions of the more versatile acetylcholine can take ove more normal neurohumoral functions.

The production of lucid intervals in catatonic schizophrenics by arecoline c eserine correlates with the observed inhibition of the CR in trained rats. Th correlation adds credence to the use of this laboratory technique for the pos sible discovery of better tranquilizing drugs for the treatment of the schizc phrenic patient.

These observations should direct our attention further to more experimer tation on the study of naturally occurring metabolites that may have a stron cerebral muscarinic action and that may be more antischizophrenic than th presently available tranquilizing drugs. The use of methyl atropine to protec schizophrenic patients from systemic symptoms while they undergo therap with acetylcholine[18, 19] would make this seemingly effective form of therap less dangerous.

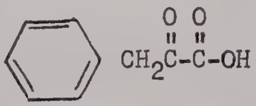

FIGURE 6. The molecular structure of phenylpyruvic acid (PPA).

The use of atropine methylnitrate to protect the peripheral portions of th body from the lethal effects of arecoline while the muscarinic action of arecolir on the brain is studied is a useful and apparently new concept based on the fa that large polar (charged) molecules do not pass the blood-brain barrier. Tl combined use of diphenhydramine methyl-iodide or Phenergan methyl sulfa with histamine in conditioned animals would disclose the effect of histamine behavior. Similarly, the study of chlorpromazine methyl iodide might d ferentiate between the central and peripheral effects of this type of compou and allow the use of larger doses of 5 HT to ascertain the effect of the latter behavior. Finally, a quaternary salt of reserpine should be compared careful with reserpine to ascertain which of the known pharmacological effects of serpine are central and which are peripheral.

Summary

Since the pharmacological effect of reserpine produces symptoms of pa sympathetic (muscarinic) stimulation, the effects of the tertiary amine mus

rinic stimulants were tested on the conditioned avoidance response of rats protected peripherally with quaternary salts of atropinelike compounds. Under these conditions, arecoline, pilocarpine, and eserine inhibit the conditioned response, and the inhibition of such responses can be prevented by atropine. Acetylcholine or methacholine do not inhibit the conditioned response. When schizophrenic patients are given subcutaneous doses of arecoline plus methyl atropine a lucid interval occurs that in several instances was more dramatic than that produced by amobarbital or 30 per cent carbon dioxide inhalations. Some patients had sobbing fits, and others attempted to escape from the interview. All patients showed more emotional responses and increased body movement after the administration of arecoline. A subcutaneous dose of 15 mg. of arecoline plus 3 mg. of methyl atropine is recommended for interview purposes. This correlation between inhibition of the conditioned response and the production of lucid intervals in schizophrenic patients confirms the use of behavioral responses in animals as a laboratory test for the discovery of new tranquilizing drugs. Research studies should be extended to include the examination of naturally occurring metabolites that have a strong central muscarinic action, since these metabolites may be more antischizophrenic than the presently available tranquilizing drugs.

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Discussion of the Paper

Paul Glees (*Oxford University, Oxford, England*): The lucid periods to which Carl Pfeiffer refers remind me of similar "lucid periods" that arise when, after prolonged treatment of human patients with reserpine, intravenous injections of amphetamine or Ritalin cause marked behavioral change within half a minute. The patient suddenly becomes talkative, excited, and more friendly. These effects wear off rapidly.

These observations were made in collaboration with David Watt, St. John's Hospital, Aylesbury, England.

NEUROPHARMACOLOGICAL STUDIES ON IBOGAINE, AN INDOLE ALKALOID WITH CENTRAL-STIMULANT PROPERTIES

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At about the turn of the century, a Frenchman by the name of Guien had the opportunity to watch an initiation to fetishism among some of the inhabitants of the Belgian Congo, Africa. According to Landrin,[1] Guien described the gross effects of chewing great quantities of roots from *Tabernanthe iboga* on the initiate as follows: "Soon his nerves get tense in an extraordinary way, an epileptic-like madness comes over him during which he becomes unconscious and pronounces words which are interpreted by the older members of the group as having a prophetic meaning and to prove that the fetish has entered him." This and other reports in the older French literature indicate that the crude extracts of *Tabernanthe iboga* caused a feeling of excitement, drunkenness, mental confusion and, possibly, hallucinations when taken in high enough doses.[2] This same plant, however, was also used widely in lower doses by the natives of the Congo to combat fatigue and tiredness when it was necessary to overcome great physical stress of any kind.[3, 4]

Dybowski and Landrin,[5] as well as Haller and Heckel,[6] were the first to isolate a crystalline alkaloid from this root, which they called "ibogaine" or "ibogine." In 1901 French pharmacologists undertook a rather detailed investigation of this alkaloid (Lambert and Heckel,[7] Lambert,[8] and Phisalix[9]), and all of them found it to have an unusual type of excitatory effect on various experimental animals such as dogs, guinea pigs, rabbits, and rats. The alkaloid also was found to have local anesthetic properties. Soon thereafter it was tried clinically and recommended as a stimulant in cases of convalescence and neurasthenia by Pouchet and Chevalier.[10] Kuborn[11] recommended it for the symptomatic treatment of trypanosomiasis. For some unknown reason, however, the drug was never widely used and was forgotten for almost 30 years. Then Rothlin and Raymond-Hamet[12, 13] took it up again and studied the effect of the alkaloid on isolated tissues and on the cardiovascular system. Delourme-Houde[14] published a comprehensive summary on the botany, chemistry, and pharmacology of *Tabernanthe iboga*, together with the results of his own investigations of the cardiovascular actions of the drug.

Ibogaine began to be of interest to us in connection with its possible phrenotropic activity. Since this drug is an indole alkaloid with allegedly central stimulant properties, it was felt that a thorough neuropharmacological and neurophysiological investigation of the drug was desirable.

Materials and Methods

Behavioral changes were studied in unanesthetized cats and dogs. In some cases, still photographs or color movies were taken. Electroshock experiments were carried out in mice (Webster strain) exclusively. A Hans Technical Associates electroshock apparatus was used in all experiments. Sixty-cycle A.C.

was applied to mice weighing 19 to 21 grams by means of ocular electrodes. The shock parameters were 60 cycles for 0.2 seconds, with currents varying from 6 to 50 mAmp.

Electrical activity of the brain was studied in cats either under anesthesia or in spinalized and decerebrate preparations. Bipolar and monopolar recordings from the cortex were made with silver ball electrodes. Stainless steel electrodes, oriented stereotactically, were employed for the pickup of subcortical potentials. The bioelectrical signals were amplified and recorded with a Medcraft 8-channel electroencephalograph.

Various reflexes and the transmission through the neuromuscular junction were investigated in chloralose-anesthetized cats (65 to 70 mg./kg. in propylene glycol, given intravenously). The monosynaptic patellar reflex was recorded as a response of the reflex movement elicited by tapping the patellar tendon rhythmically. The polysynaptic reflex arc was tested by electrical stimulation of the afferent part of the tibialis nerve and by recording the height of contraction of the ipsilateral anterior tibialis muscle (flexor reflex). In the same preparation the peripheral end of the cut radial nerve was stimulated intermittently, and the contraction of the isolated radial muscle was measured. The blood pressure in the carotid artery was registered by means of a mercury manometer.

Ibogaine hydrochloride (Bogadin T.M.) was injected in a 1 per cent aqueous solution in all experiments by various routes of administration.

Results

Effects on spontaneous behavior in unanesthetized animals. The effects of ibogaine on spontaneous behavior had been studied by various French authors, particularly in dogs.[8, 9] It was possible to confirm their observations in unanesthetized cats and dogs that had received doses of ibogaine hydrochloride (2 to 10 mg./kg.). In view of the fact that these experiments were performed more than 50 years ago and may not be known generally, it is considered appropriate to describe a typical experiment in a cat. Upon intravenous injection, the onset of drug activity was almost immediate. The cat became markedly excited, the pupils dilated, salivation, partial piloerection, and tremors started that gradually developed into an actual picture of rage. Usually the animal remained in one place, slightly shivering, the tail outstretched, while making hissing sounds as if trying to scare off an imaginary object (FIGURE 1). Often the cat tried to move toward a corner, hide there, and bury its head in it. Sometimes the animal approached a corner and tried to climb up the walls, apparently attempting an escape. Ataxia was noted. At the height of the excitatory phase, depending upon the dose, the animal exhibited a peculiar type of clonic extension of the hind limbs and front legs, causing spreading of the extremities in all directions with the abdomen on the floor. Mewing was frequently observed at that stage. The respiration appeared to be rather fast at times. Salivation was usually present. No urination or defecation occurred during the phase of rage or fear. The maximum degree of excitement was usually reached within a period of 10 to 20 minutes

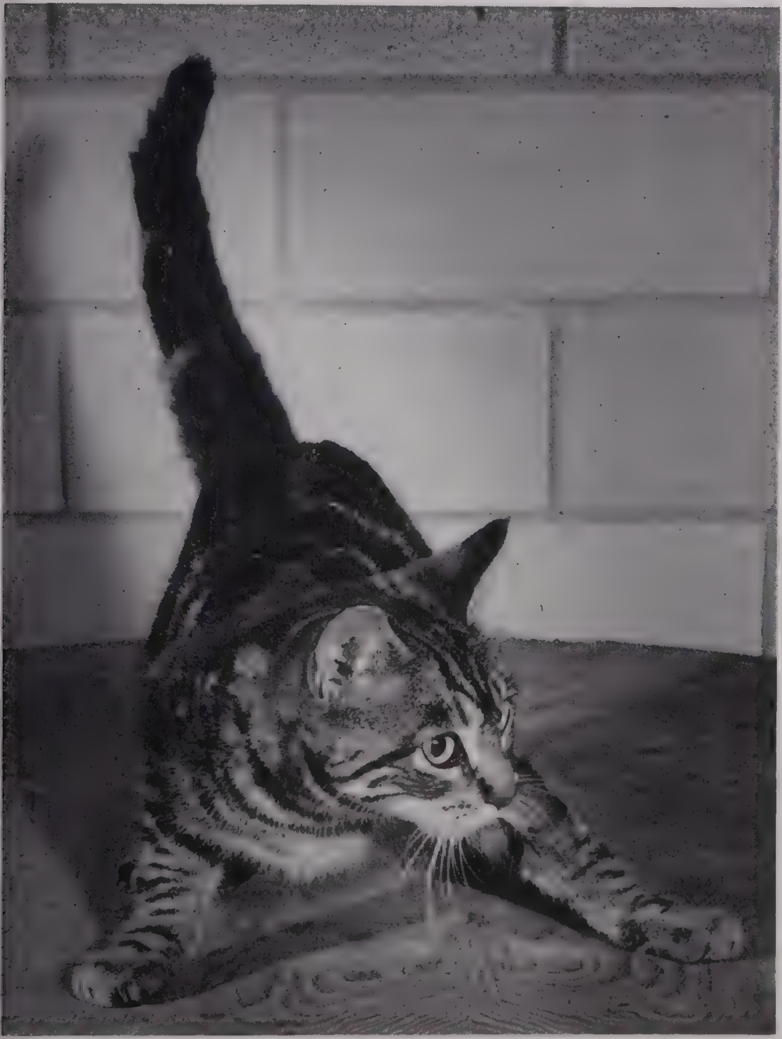

FIGURE 1. The effect of ibogaine hydrochloride (5 mg./kg. given intravenously) in an unanesthetized cat. Note the size of the pupil, which is wide, despite the presence of intense artificial illumination. Note also the apprehension and alertness, the outstretched tail, and the peculiar position of the legs.

following the intravenous injection, after which the excitability gradually subsided. After 1 to 2 hours the animal appeared to be normal again.

These symptoms indicated that ibogaine hydrochloride had a central stimulating effect, the nature of which, however, was not clear. The unusual pat-

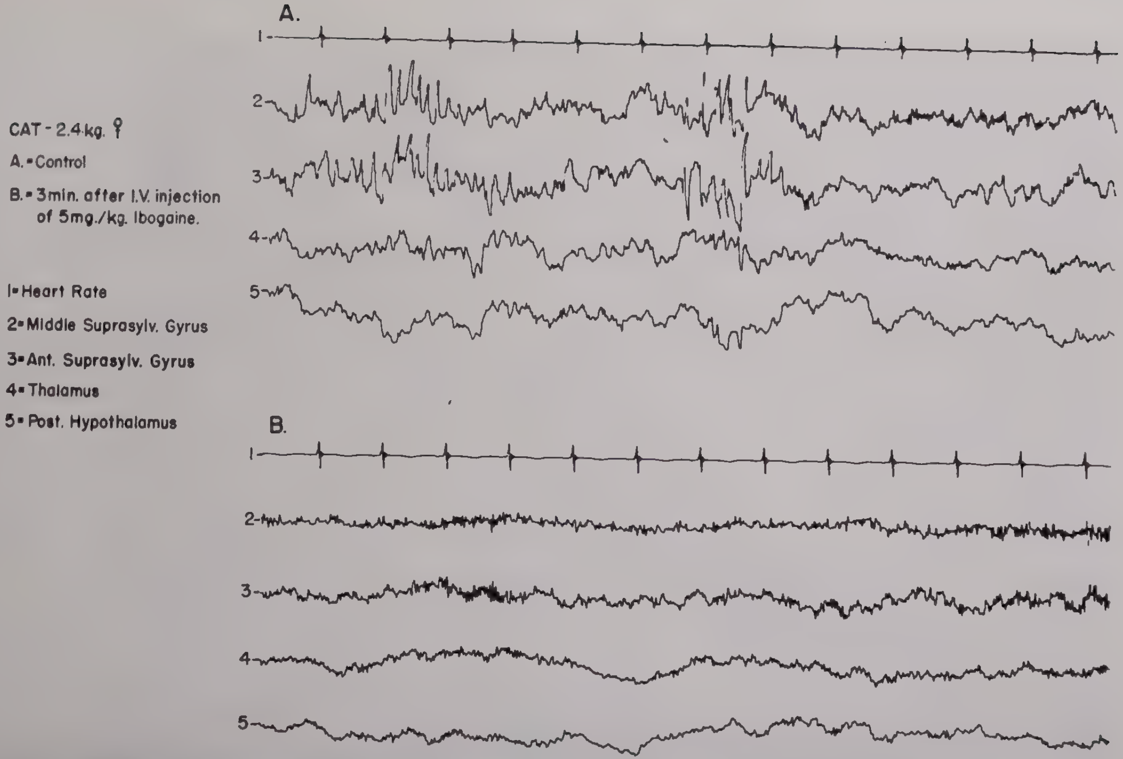

FIGURE 2. The effect of ibogaine on the electrical activity of the brain of the cat (spinalized at C), shown by electrocorticograms before and after the injection of ibogaine hydrochloride. Spindles that can occur under normal conditions in high-spinalized preparations (tracing A) are abolished completely by the intravenous injection of ibogaine hydrochloride, which causes fast activity of low amplitude, as seen in tracing B.

tern of muscular activity consisting of an increase in extensor tone associated with ataxia in higher doses led us to investigate the electrical activity of the brain and reflex mechanisms, as well as susceptibility to convulsions.

Electroencephalographic studies. Electrocorticograms of cats with *cerveau isolé* and *encéphale isolé* preparations, as well as curarized animals, showed a typical arousal syndrome after doses of ibogaine hydrochloride (2 to 5 mg./kg.) given intravenously. The electrocorticogram that, under normal conditions, depending upon the type of preparation, was characterized by slow-frequency

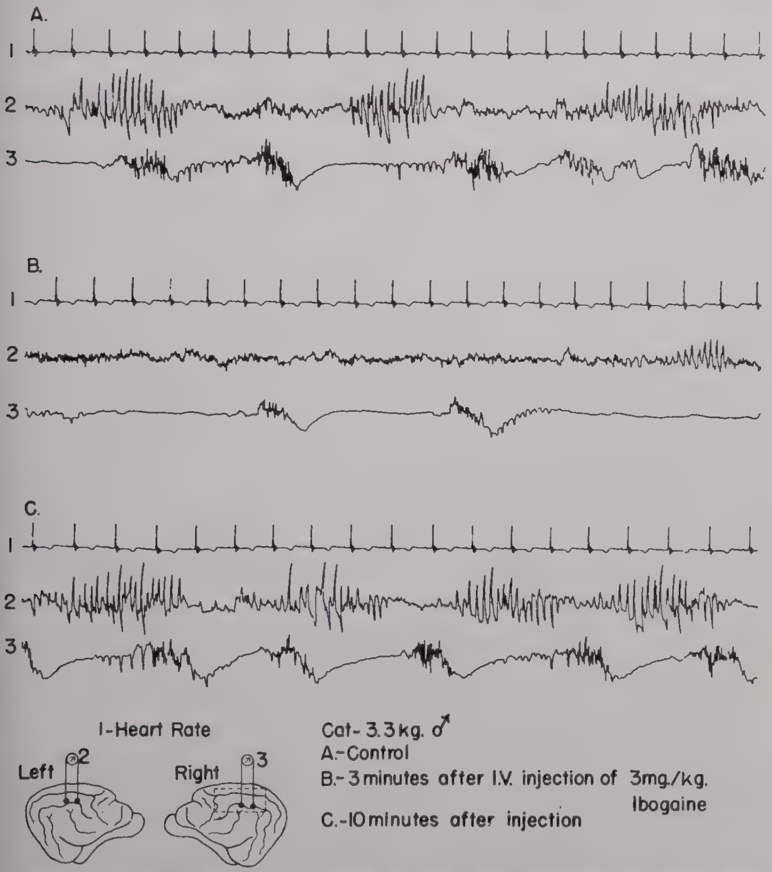

Figure 3. Electrocorticograms of a cat preparation (intercollicular decerebration and undercut cortex), representing the electrical activity over the intact cortex (tracing 2) and the undercut cortex (tracing 3), simultaneously with the heart rate (tracing 1).

(A) The electrical activity of the brain, showing spindle formation of the intact cortex and bursts of fast activity characteristic of the electrical pattern over the undercut cortex.

(B) The alerting pattern following the injection of ibogaine hydrochloride. Note that the spindle formation is not suppressed completely, and that the spontaneous-burst activity in tracing 3 is reduced somewhat.

(C) Rapid recovery from the relatively large dose of ibogaine hydrochloride in animals with intercollicular decerebration.

and high-amplitude activity associated with spindle formation, showed a very marked change toward fast activity of low amplitude (FIGURE 2). The electroencephalographic pattern obtained after ibogaine resembled closely that obtained after direct stimulation of the reticular formation. The low-voltage, high-frequency activity persisted for a period of from 10 to 20 minutes, depending upon the dose applied. After 30 minutes to 1 hour, full recovery usually could be observed.

When higher transsections of the brainstem of the cat were performed, such as intercollicular decerebration, the alerting reaction was still observable after ibogaine (FIGURE 3), though usually to a lesser degree. In some preparations with cortical flaps only a slight or no reduction (FIGURE 3) of spontaneous burst activity characteristic for these preparations occurred.

Because of the similarity between the alerting pattern seen after ibogaine

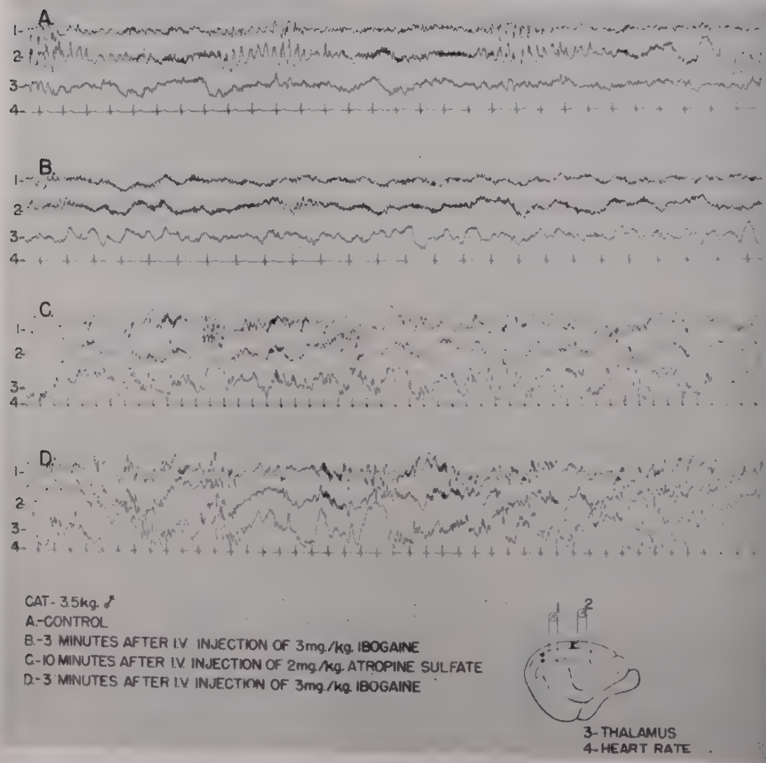

FIGURE 4. Electrocorticograms of a cat with high-spinalization. (A) Activity during control period; (B) activity 3 minutes after the injection of 2 mg./kg. of ibogaine hydrochloride; (C) 10 minutes after the injection of 2 mg./kg. of atropine; and (D) the pattern of activity following the injection of ibogaine hydrochloride after pretreatment with atropine. Note the complete absence of the alerting reaction following the administration of atropine.

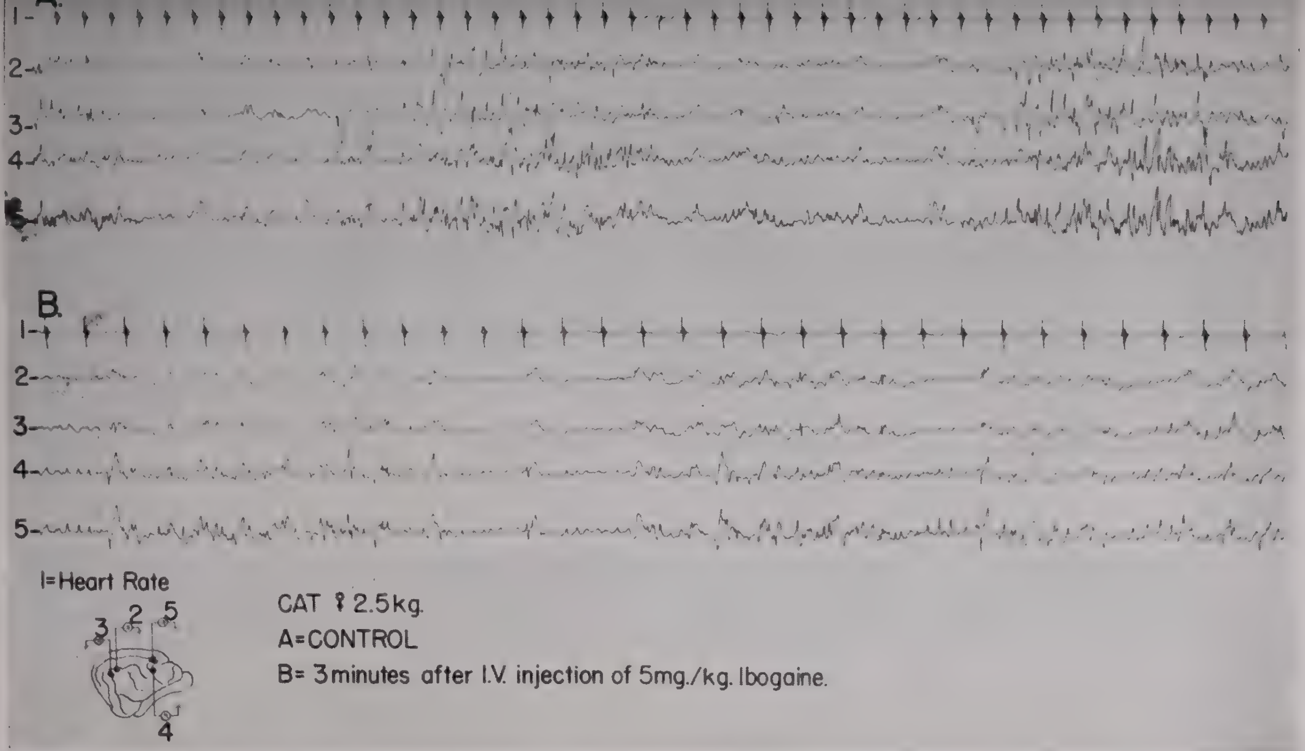

FIGURE 5. The effect of ibogaine on the brain of a cat under anesthesia with diallyl barbituric acid. (A) Electrocorticograms showing the normal sleeping pattern under barbiturate anesthesia. The effect of ibogaine hydrochloride is demonstrated in record B. Note the reduction in amplitude of the electrical activity of the brain, including a clear-cut reduction of the amplitude and frequency of the barbiturate spindle.

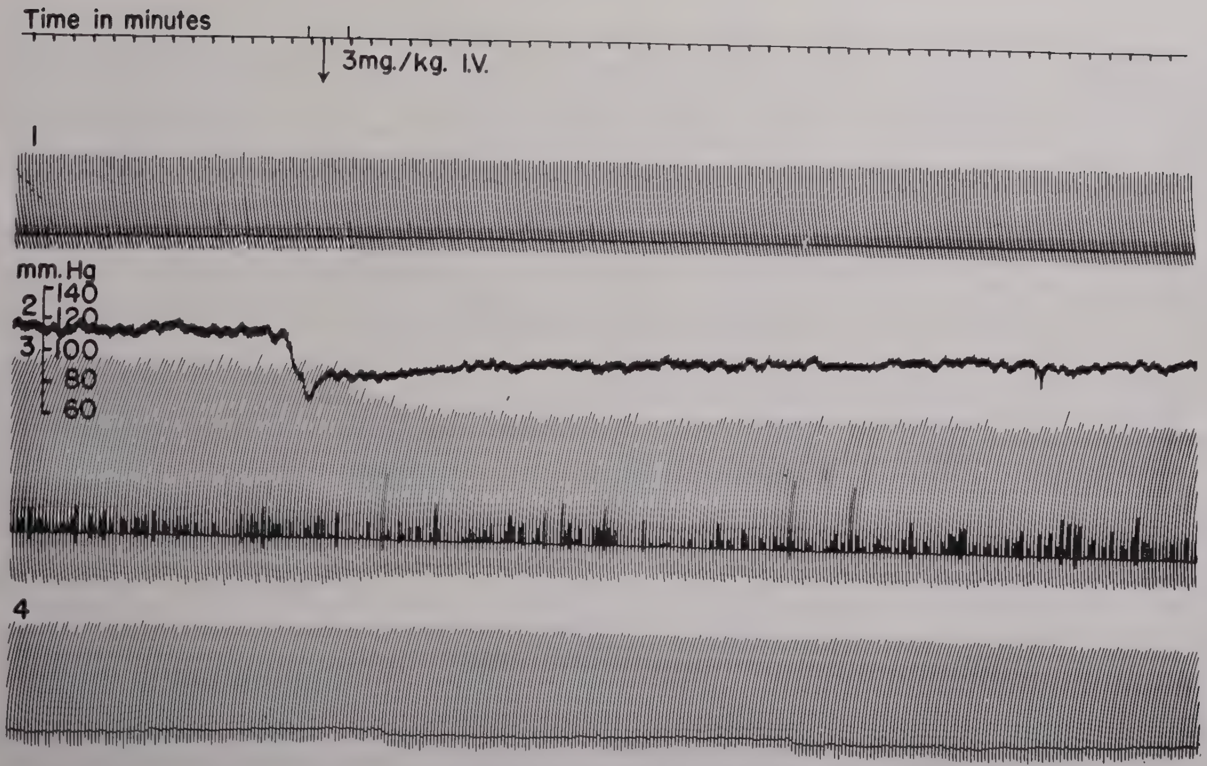

FIGURE 6. The effect of ibogaine hydrochloride on the spinal reflexes of a cat anesthetized by chloralose: (1) polysynaptic reflex; (2) blood pressure; (3) knee jerk; and (4) nerve-muscle preparation. Note the reduction in muscle contraction following the tapping of the patellar tendon and the absence of any effect on the

TABLE 1

PERCENTAGE OF 3 GROUPS OF 10 MICE TREATED WITH IBOGAINE HCl (10 MG./KG. S.C.), SHOWING FULL SEIZURES FOLLOWING ELECTROSHOCK

Minutes after injection	Current intensity		
	6 mAmp.	12.5 mAmp.	18 mAmp.
Control	0	70	100
45	0	20	90
90	0	0	90
180	0	20	90
300	0	80	100

The effect of ibogaine hydrochloride on the electroshock-seizure threshold in mice. Ibogaine hydrochloride increases the threshold to electroshock seizure in this species, showing a maximum effect 90 minutes after the subcutaneous injection of the drug. The anticonvulsant property of ibogaine hydrochloride is weak, and the drug does not give significant protection when supramaximal stimuli are applied.

and after electrical stimulation of the reticular formation, atropine in doses of 2 mg./kg. was given to 3 cats previous to ibogaine injection. As reported by Rinaldi and Himwich,[15] atropine blocked the cholinergic mechanism of the reticular ascending system. Following these larger doses of atropine, ibogaine failed to produce an arousal syndrome (FIGURE 4).

In the experiments done under barbiturate anesthesia, a reduction of amplitude of barbiturate spindles and a general decrease in amplitude of the EEG was noted (FIGURE 5).

Studies on reflexes. In view of the rather marked increase in muscular tone, a thorough study of various types of reflexes was undertaken in anesthetized, decerebrated, and highly spinalized cats. The only consistent finding was a slight reduction of the monosynaptic knee-jerk reflex that followed intravenous injection of ibogaine in doses of 2 mg./kg. and higher (FIGURE 6). Polysynaptic reflex arcs did not appear to be affected at all, even in high doses. Also, there was no apparent influence on the effect on neuromuscular transmission.

Electroshock experiments. The effect of ibogaine on the threshold and electroshock seizure pattern was studied in mice according to techniques described by Swinyard, Brown, and Goodman.[16] TABLE 1 summarizes the results of an experiment in 30 mice, demonstrating the increase in the threshold to electroshock after ibogaine (10 mg./kg.) given subcutaneously. The anticonvulsive effect lasted more than 3 hours.

In a second series of experiments, electric shock of approximately 4 times the threshold value (50 mAmp.) was delivered to a total of 30 mice. Ibogaine hydrochloride in doses of 10 and 20 mg./kg. prolonged the electroshock latency significantly without affecting the other components of the seizure pattern. It was noted, however, that when ibogaine hydrochloride was administered previous to the shock, the mice were walking around almost immediately after the termination of the clonic phase.

Discussion

The results presented in this paper indicate that ibogaine hydrochloride is an alkaloid with distinct central-stimulating properties. Following high doses,

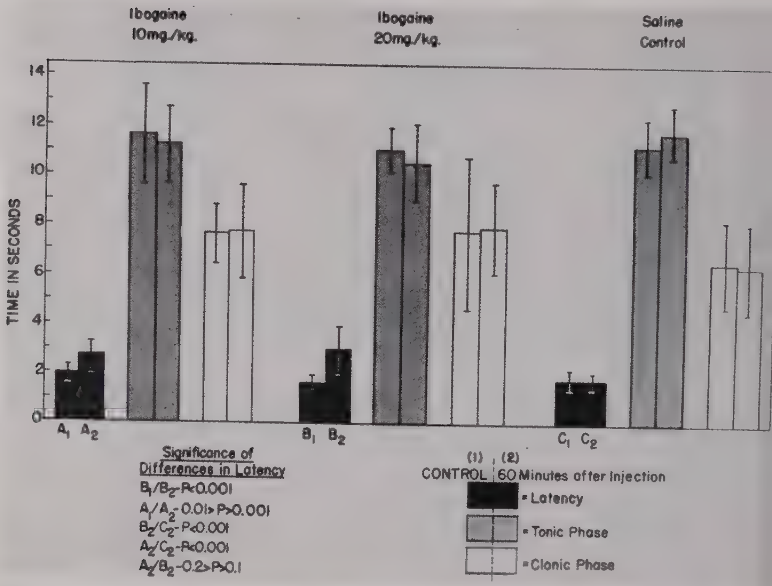

FIGURE 7. The influence of various doses of ibogaine hydrochloride on the electroshock-seizure pattern in mice. The only change visible is the increase in latency that is highly significant (P lower than 0.001). This is in accord with the increase of the electroshock-seizure threshold.

this central excitatory effect is accompanied by reactions of apprehension and fear, and the animals have the desire to hide or to escape from a situation which is obviously frightening them. When they are unable to escape, the main reaction of the animals may consist of aggression toward real or imaginary opponents. Ataxia presents itself regularly after higher doses of the drug.

One point of attack of the drug appears to be the ascending reticular formation of the brainstem, stimulation of which causes an arousal syndrome (Magoun[17]). Three of our findings point in this direction:

(1) The EEG response to electrical stimulation of the reticular formation[17] resembles closely the EEG effect observed after intravenous injection of ibogaine hydrochloride.

(2) High decerebration (*cerveau isolé* preparation) that eliminates the influence of a major portion of the reticular formation causes a reduced and shortened alerting response after ibogaine hydrochloride injection.

(3) The alerting response normally following the administration of ibogaine hydrochloride can be abolished by atropine. This finding indicates the involvement of a cholinergic mechanism in the central-stimulating effect of ibogaine hydrochloride. This is in accord with studies of Vincent and Sero,[18] who reported choline esterase inhibition by ibogaine.

In view of the central-stimulant nature of the drug and the extensor spasms of the extremities often associated with it, the minimal effect on reflex activity is somewhat surprising. The mildly depressant action on the monosynaptic reflexes is rather nonspecific. It may be observed after dosage with a variety of drugs, epinephrine for example, that reactions of this type are largely dependent upon the type of preparation and anesthesia used (Sigg, Ochs, and Gerard[19]). Our studies exclude a strychninelike component of ibogaine hydrochloride, however, and a direct stimulating action on the striated muscle or neuromuscular conduction is also unlikely.

A surprising effect of ibogaine as a central stimulant is the increase of the threshold to electroshock which is also reflected in a significant increase in electroshock latency. Everett, Toman, and Smith[20] and Toman, Everett, and Smith[21] have recently indicated that some central stimulants and excitants, paradoxically, may increase electroshock latency in mice. Ibogaine hydrochloride, therefore, must be included in a group of central stimulants that have weak but definite anticonvulsant properties.

Summary

(1) Ibogaine hydrochloride is an indole alkaloid with characteristic stimulant properties.

(2) Electroencephalographic experiments indicate that 1 point of attack of this alkaloid lies in the ascending reticular formation.

(3) Reflex studies exclude a strychninelike component in the mode of action of ibogaine hydrochloride.

(4) In spite of its central excitatory action, ibogaine hydrochloride increases the electroshock threshold and prolongs latency in mice.

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Part V. Psychotherapeutic Drugs: Neurophysiological and Biochemical Effects

A REVIEW OF THE NEUROPHYSIOLOGICAL EFFECTS OF PSYCHOTHERAPEUTIC DRUGS

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The effects of the most widely used psychotherapeutic agents were discovered only a few years ago by observations on psychiatric patients and not by experimental studies on the central nervous system. An experimental neuropharmacological basis for an explanation or an understanding of the effects on the psyche and behavior is not at hand at this time, although great efforts are being made by a large number of investigators in many laboratories, working from very different approaches and under widely varying premises. A review at this time could be hardly anything but an enumeration of these efforts, and the space available would be far too small even for such an enumeration.

Not wanting to shirk my assignment, I shall serve the topic better, I believe, by confining myself to the search for certain trends in neurophysiological studies of these drugs and by endeavoring to discern similarities and dissimilarities in their effects, under certain conditions, on patterns of organization in the central nervous system. This seems to me all the more appropriate since there is as yet only little agreement among the various laboratories as far as methodology is concerned. Indeed, suitable methods for the investigation of these drug effects are in the developmental stage, and neurophysiological observations in which more than 1 drug has been studied under comparable experimental conditions are rare.

Among the drugs that are used by psychiatrists in patients with anxiety neuroses or psychoses are those of the mephenesin type. A drug with a mephenesinlike type of action is characterized by its ability to depress internuncial neurons in the spinal cord and in the bulbar reflex regulating centers. This ability is evidenced by depression and loss of the withdrawal reflexes, of flexor reflexes, of spinal reflexes elicited by contralateral stimulation and, in short, of all motor reflexes of polysynaptic nature (Berger, 1949). These drugs suppress the facilitation and the inhibition of motor reflexes obtained on stimulating certain areas in the reticulobulbar formation (Henneman, Kaplan, and Unna, 1949; Kaada, 1950; King and Unna, 1954). Their depressant effect on multineuronal pathways can be demonstrated particularly well by the suppression of the secondary spike potentials derived from the ventral root on stimulation of the corresponding dorsal root in Lloyd's segmental preparation (Hennemann, Kaplan, and Unna, 1949). That monosynaptic arcs may not be wholly resistant to the depressant action of drugs of this class was shown recently by Latimer (1955). Also, it has been shown repeatedly that these drugs, in larger doses, have effects on suprasegmental structures in the brain (Funderburk, King, and Unna, 1953b). Anesthetic or analgesic effects are not seen with doses that depress polysynaptic spinal and bulbar reflexes, however. In addi tion, these compounds exhibit more or less pronounced anticonvulsive actior They antagonize not only convulsions following the administration of strych nine but also, to a lesser extent, those produced by metrazol, and they alte the pattern of electroshock-induced seizures. These drugs have little if an effect on autonomic functions, either by peripheral action or on central reg ulations. The emergence of these drugs, which we like to classify for reasons o their neuropharmacological action as interneuron depressants, began with th observations of Goodman (1943) on benzimidazole and the studies on mephene sin by Berger and Bradley (1946). The neuropharmacological spectrum of ac tion is common to mephenesin, mephenesin carbamate, a 2,2-diethyl-1,3 propanediol or Prenderol (Funderburk and Unna, 1953), and to the recentl introduced 2-methyl 2-n-propyl-1,3-propanediol dicarbamate or meprobamat (Berger, 1954; Hendley, Lynes, and Berger, 1955); to atrolactamide (King 1953); and also to benzazoles (Domino, Unna, and Kerwin, 1952; Funderburk Domino, and Unna, 1953a); benzimidazole, benzothiazole, and benzoxazole of which 1 derivative, 2-amino-5 chlorobenzazole, was introduced in 195 (Marsh, 1955; Kamijo and Koelle, 1955). Available drugs of this type thu are either derivatives of glycol or of benzoxazole, and their number undoubtedl will grow. It will be interesting to learn whether still other compounds o different structure are endowed with similar neurophysiologic action. This should not be difficult to establish, because the last decade has provided the student with a more or less accepted methodology for systematic investigation of the neuropharmacological action of this type.

Turning toward drugs such as reserpine and chlorpromazine, which profoundly affect the psyche and behavior, particularly of agitated psychoses, we discern at once that their spectrum of action, as far as it is explored today, is totally different from that of the interneuron depressants, and that they differ markedly from each other in some of their neurophysiological effects. I shall restrict my remarks to reserpine and chlorpromazine, because the neurophysiological evidence for the effects of other more recently introduced drugs is still too scanty.

We should not overlook the fact that originally both of these agents, reserpine and chlorpromazine, were isolated or synthesized respectively by the pharmaceutical chemists for their effects on autonomic functions, and that only later were the tranquilizing effects, to which they owe their great popularity, discovered in patients. It reminds one of the similar development of lysergic acid diethylamide (LSD) from alkaloids with predominantly autonomic effects, and also of the accidental discovery of mood-changing effect of amphetamine, a compound that was designed originally as a sympathomimetic vasoconstrictor.

The autonomic effects of reserpine, hypotension and bradycardia, are independent of the peripherally operative mechanisms in the autonomic nervous system. Reserpine alters the autonomic functions by impinging on the regulations of these functions within the central nervous system. This is borne out by studies on the hypothermic effects of reserpine, on its emetic and antiemetic effects, by studies on bradycardia and, most extensively, on centrally mediated espiratory and vasomotor reflexes. The evidence for these observations has een reported fully elsewhere (Yonkman, 1955). The search for the site of this ction of reserpine revealed that direct stimulation of the midbrain vasomotor enters and of medial and ventromedial sites of the hypothalamus still elicited naltered responses after the administration of reserpine. The experiments f Schneider (1955) and of Harrison and Goth (1956) adduced no direct evi- lence for an elevation in the threshold of sensitivity of these autonomic centers o electrical stimulation. By the process of elimination these experiments led o the postulate that reserpine blocks the afferent pathways of these centers entrally, thus attenuating their stimulation and, in particular, those of the ympathetic centers of regulation. One may but speculate that reserpine auses an increase in cortical inhibition on diencephalic structures, or that eserpine activates inhibitory central sites that may be located rostrally to the nferior colliculi. Very recently, however, Bhargava and Borison (1955) and orwitz and Wang (1956) presented contradictory evidence. The experiments f these investigators demonstrated that reserpine does depress the pressor re- ponses from brainstem vasomotor centers, and that the medullary vasomotor enters are susceptible to reserpine even after midcollicular decerebration.

The explanation that reserpine causes activation of inhibition in areas that re as yet not localized precisely stands in contrast to the findings of increased acilitation by reserpine at other sites. Thus reserpine in large doses facilitates he patellar reflex of spinal animals and increases the monosynaptic potential btained in the segmental preparation of Lloyd (Schneider *et al.*, 1955). Re- erpine lowers the threshold to electroshock, enhances the tonic extensor re- ponse to pentamethylenetetrazol (Metrazol), facilitates the spread of seizures, nd prolongs the duration of seizures (Jenney, 1954; Chen, Ensor, and Bohner, 954). It also can cause seizure activity in the rhinencephalon (Killam and illam, 1956). Furthermore, reserpine antagonizes the effects of morphine Schneider, 1954).

The effects of reserpine on the electroencephalogram (EEG) are usually ight and variable. The EEG of spinal cats in which slow-frequency, high- mplitude potentials predominate shows, after reserpine, an increase in low- oltage, high-frequency activity. Thus it was a disappointment to find that e responses in the electrocorticogram obtained by direct stimulation or by eripheral stimulation of the reticular activating system were not altered signifi- ntly by reserpine. Killam and Killam (1956) failed to demonstrate a change the threshold of this area or in the thalamocortical recruiting circuits in cats. n the other hand, Rinaldi and Himwich (1955) obtained increased facilitation the arousal response in rabbits with large doses of reserpine.

In contrast to reserpine, chlorpromazine alters the autonomic functions, in rt, by acting on the peripheral autonomic structures. Chlorpromazine has a ild adrenergic-blocking effect evidenced by decrease and, under certain con- tions, by reversal of the vasoconstrictor effects of injected epinephrine. It not clear whether this effect is caused by blockage of adrenergic receptor sites the smooth muscle, because the same doses of chlorpromazine do not decrease e vasocontrictor response to norepinephrine, but enhance and prolong it

(Martin, 1956). Claims that chlorpromazine possesses ganglionic blockin action have not been confirmed in experiments on the cervical sympatheti ganglion (Holzbauer and Vogt, 1954). The bradycardia following the ac ministration of chlorpromazine is obtained in spinal or stellectomized, vagoto mized animals, and is thus of peripheral origin.

The greater part of the hypotensive action of the drug is due to its effects o the central autonomic pathways. This had been shown by many studie particularly those of Courvoisier *et al.* (1953), and has been confirmed recentl in cross-circulation studies in dogs (Krause and Schmidtke-Ruhnau, 1955 Other important central autonomic characteristics of chlorpromazine are it antiemetic and hypothermic effects. The site of action of the antiemetic actio of chlorpromazine lies in the emetic trigger zone of Borison and Wang in th postrema area. Chlorpromazine, in small doses, blocks apomorphine an hydergine emesis (Brand *et al.*, 1954; Glaviano and Wang, 1955). In large doses, chlorpromazine also depresses the medullary emetic center, and it pre vents emesis by other centrally acting drugs that operate directly on this cente

In support of a direct depressant action of chlorpromazine on the thermo regulatory center, chlorpromazine abolishes the pyretic effect of vaccines an causes hypothermia in animals maintained at lowered environmental tempera ture without causing a significant change in their oxygen consumption.

Studies on the action of chlorpromazine on responses elicited from hypo thalamic and medullary pressor areas have not yielded unequivocal results In decorticate cats, small doses have been found to block the pressor respons (Dasgupta and Werner, 1954), while they were ineffective in unanesthetize and curarized cats and in animals anesthetized with chloralosane (Gunn Jouvet, and King, 1955). Under these conditions the animals required rela tively large doses of chlorpromazine to abolish the pressor effects.

Available evidence for other possible sites of action of chlorpromazine in th central nervous system further differentiates the mechanism of action of thi drug from that of reserpine. Chlorpromazine is not known to facilitate moto reflexes. It does not alter either monosynaptic or polysynaptic potentials i Lloyd's preparation. It does not lower the threshold of convulsions. I potentiates significantly the effects of a large number and variety of hypnotic and analgesics.

The effects of chlorpromazine on the EEG in spinal or curarized and unanes thetized animals are characterized by a general decrease in frequency and b the appearance of 8- to 12-cycles-per-second bursts. These bursts are not wel formed, and they differ from the symmetrical spindles that are seen with bar biturates. In addition, there appear 7- to 10-cycles-per-second waves that hav a more gradual ascending limb, as well as 1- to 3-cycles high-voltage potential These results have been obtained in different laboratories with doses of chlor promazine from 1 to 10 mg./kg. (Hiebel, Bonvallet, and Dell, 1954; Longo, vo Berger, and Bovet, 1954; Rinaldi and Himwich, 1955; DeMaar and Marti 1956). With smaller doses and, occasionally, with a dose as high as 5 mg./kg the decrease in frequency of the EEG potentials is less apparent.

Activation of the EEG by electrical stimulation of the bulbar-reticular a vating system or by auditory or peripheral nerve stimulation can be abolished y chlorpromazine. These effects, reported by different investigators, have een obtained in rabbits and cats (Rinaldi and Himwich, 1955; Longo, von erger, and Bovet, 1954). Furthermore, the arousal response induced by epi- ephrine is depressed or markedly modified in its pattern by chlorpromazine. lthough other investigators found these effects of chlorpromazine less striking, is tempting to interpret the effects of chlorpromazine on the activating system s evidence for its predominant site of action. Indeed, such a concept with r-reaching implications has been advanced by Dell and his associates (Hiebel, onvallet, and Dell, 1954). Further experimentation and careful analysis of e effects of other than direct electrical stimulation of the activating system re necessary to support the explanation offered by these authors. Further and ore conclusive evidence for effects on afferent pathways, in particular, seems ecessary.

In summarizing, it is evident that convincing neurophysiological evidence for e action of reserpine and of chlorpromazine is not yet at hand. It appears this reviewer that the psychotherapeutic effects of these substances, as well s those of all other potent psychotomimetic agents, are entwined with pro- ounced action on autonomic regulations. The much sought-for neurophysio- gical evidence would thus have to fit and to explain both the tranquilizing *and* e autonomic effects of these drugs. It appears more than likely that the fects of these agents alter the response and responsiveness to chemical media- on in the central nervous system, and that further intensive studies on the europhysiological effect of mediators, whose presence in brain tissue has been tablished, are needed. Unfortunately, our knowledge of the effects of the aturally occurring pressor amines on the function of the brain is still meager.

The challenge presented by the psychotomimetic drugs will accelerate the ac- uisition of facts and knowledge on the transmission of impulses in the brain, cts that are being gathered by the neurochemist, neurophysiologist, and neuro- armacologist alike.

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Discussion of the Paper

JURG A. SCHNEIDER (*CIBA Pharmaceutical Products, Inc., Summit, N. J.*): ince Klaus Unna presented our theory of the action of reserpine in his review nd discussed it in connection with the more recent findings of Bhargava and orison on the one hand and Goth and Harrison on the other, I should like to escribe our own theory in more detail. I regret very much that neither Bharava and Borison nor Goth and Harrison are here to discuss their reports.

With regard to the experiments of Bhargava, who found a diminution of the esponsiveness of the medullary vasomotor centers by reserpine, we have one bjection. In his experiments, a decrease in responsiveness to medullary imulation was shown that lasted from half an hour to an hour and was fully ecovered after that period of time. The blood pressure effect of reserpine is not omplete, however, before at least 1 to 2 hours have elapsed. Thus it seems ery unlikely that the blood pressure reducing effect of reserpine can be exlained on the basis of Bhargava's findings.

Goth and Harrison, on the other hand, showed in their experiments with ypothalamic stimulation that the response to stimulation following the adinistration of reserpine was undiminished or even augmented. In this respect eir findings are identical with ours, but they interpret their results differently. ccording to their concept, the depressant effect of reserpine on the hypothalamic rea is masked by the potentiating effect of reserpine on the epinephrine and orepinephrine response. The threshold response to hypothalamic stimulation ppears to be unchanged by reserpine, however, and a threshold change would ave to be expected if a direct dampening effect of reserpine on the hypoalamic centers were to be assumed.

We have postulated that the mechanism of vasomotor depression might be an direct one, and more recent findings by Killam and MacLean have shown that eserpine has a definite effect on the limbic system. It is too early to evaluate nally the significance of these findings, but they are a definite indication that e reserpine drug effect may involve structure above the hypothalmic level at may be responsible for some of its pharmacodynamic effects.

THE EFFECTS OF PSYCHOTHERAPEUTIC COMPOUNDS ON CENTRAL AFFERENT AND LIMBIC PATHWAYS*

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Evidence of behavioral tranquilization following the administration of rese pine and chlorpromazine to experimental animals and to man led to speculati that the drugs might specifically depress the ascending reticular-activating sy tem (Lehmann and Hanrahan, 1954; Terzian, 1952; Delay and Deniker, 195 Sigwald and Bouttier, 1953). This extralemniscal afferent system involvi the reticular formation of the brainstem was first described by Moruzzi a Magoun (1949), and its importance in the maintenance of normal wakefulne and alertness has been clearly demonstrated by numerous studies (Magou 1950a, 1950b, 1952). Not only does stimulation of the reticular formation le to cortical desynchronization and behavioral arousal (Moruzzi and Magou 1949; French *et al.*, 1952), but lesions destroying this area or cutting its proje tions anteriorly (Lindsley *et al.*, 1949; Lindsley *et al.*, 1950; French and M goun, 1952) induce slow synchronous electroencephalographic (EEG) pattern spindles, and behavioral somnolence like that following midbrain secti (Bremer, 1935). Lesions of the direct lemniscal pathways by which senso impulses reach the cortex do not produce similar changes, nor do they block t EEG desynchronization induced by reticular stimulation. Furthermor stimulation of certain portions of the diffuse thalamic projection system d scribed by Morison and Dempsey (1943; see also Dempsey and Morison, 194 and by Jasper (1949; see also Jasper and Droogleever-Fortuyn, 1946) with hig frequency pulses has been shown to induce EEG desynchronization in wid spread cortical areas (Starzl and Magoun, 1951). Low-frequency stimu applied to the same nuclei cause recruiting responses reminiscent of the EE spindle activity noted with drowsiness or light sleep. The thalamic syste has been considered widely as the pathway by which reticular impulses reach t cortex (Starzl and Magoun, 1951; Rinaldi and Himwich, 1955b; Himwich an Rinaldi, 1955–56), although anatomical (Starzl *et al.*, 1951), physiologic (Jung and Riechert, 1954), and pharmacological evidence (Domino, 1955; Jun and Riechert, 1954; King, 1954) suggest that the reticular and thalamic sy tems are capable of independent function.

Recent neuropharmacological investigations based on current knowledge these corticipetal pathways have attempted to localize and characterize th action of tranquilizing agents in the central nervous system. Results obtaine in different species and with various techniques have differed considerably, a reviewed elsewhere in this publication by K. R. Unna.

In our studies of the unanesthetized, curarized cat (Killam and Killam, 1956 1956b), the effects of pharmacological doses of chlorpromazine (up to 8 mg./kg on the threshold and duration of EEG arousal following reticular stimula tion were much less impressive than those of barbiturates at 5 to 10 mg./k ıresholds in the thalamus were even more resistant to the compound. Re-rpine at 100 γ/kg. did not alter the EEG arousal mechanisms studied under ese conditions. More positive effects were noted in the limbic system. Sei-ıres following stimulation of the afferent inflow to the hippocampus via the rnix were enhanced by reserpine and depressed by chlorpromazine.

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The experiments reported here were undertaken in an attempt to explain e observable behavioral depression produced in cats by doses having such ıall effects on the pathways studied in acute experiments. The influences reserpine and chlorpromazine were tested on the same central pathways in ɔncurarized cats having chronically implanted electrodes for stimulation and cording. By such studies it was possible to correlate electrophysiological ıd behavioral responses to stimulation of the reticular activating system, the ffuse thalamic projection system, and the rhinencephalic system.

Methods

Under pentobarbital anesthesia, electrodes oriented stereotaxically were ıplanted at various sites in the brains of adult cats. Deep electrodes were polar nichrome wire insulated except for ½ mm. at the tips. The interelec-ɔde distance was about ½ mm. Cortical recordings were taken from screws st penetrating the skull. The electrodes were connected to the various ter-inals of a socket secured to the skull. After a week to 10 days, when the skin ıs well healed around the socket, recording and stimulation could be carried t through a cable plugged into the socket for the test period. The animal ıs free to move about, and behavioral observations were made through a 1-way irror. Thresholds for EEG and behavioral changes were measured only when e animals appeared to be in natural sleep. The criteria for such sleep were gular respiration, closed eyes, sleeping position, relaxation, and absence of ɔvement. Square wave stimuli were given from a Grass stimulator with an ɔlation unit of 0.1 msec. duration. Stimuli causing EEG arousal were /second, and those to the limbic system were 150/second. All were moni-red periodically for voltage and amperage to check the electrode resistance. ecordings were made on an 8-channel Offner electroencephalograph. Thresh-ds for the various phenomena obtained over periods ranging from 2 to 7 ɔnths showed surprisingly little variability. Reserpine phosphate*, chlor-omazine hydrochloride†, and pentobarbital sodium were each given in several ses to each cat during these periods.

Results

Effects on EEG activation and behavioral arousal following reticular stimulation. ıe responses to stimulation of the midbrain reticular formation in chronic imals were similar to those described by earlier investigators. When muli of threshold voltage were applied there was a fall in the amplitude of the EG and an increase in its frequency. Typical effects following 3 and 4 v. mulation in cat CI 13 are illustrated in FIGURES 1, 3, and 4. A slight increase

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* Supplied through the courtesy of Jurg Schneider, Research Laboratories, CIBA Pharmaceutical Products, ., Summit, N. J.

† Supplied through the courtesy of E. J. Fellows, Smith, Kline & French Laboratories, Philadelphia, Pa.

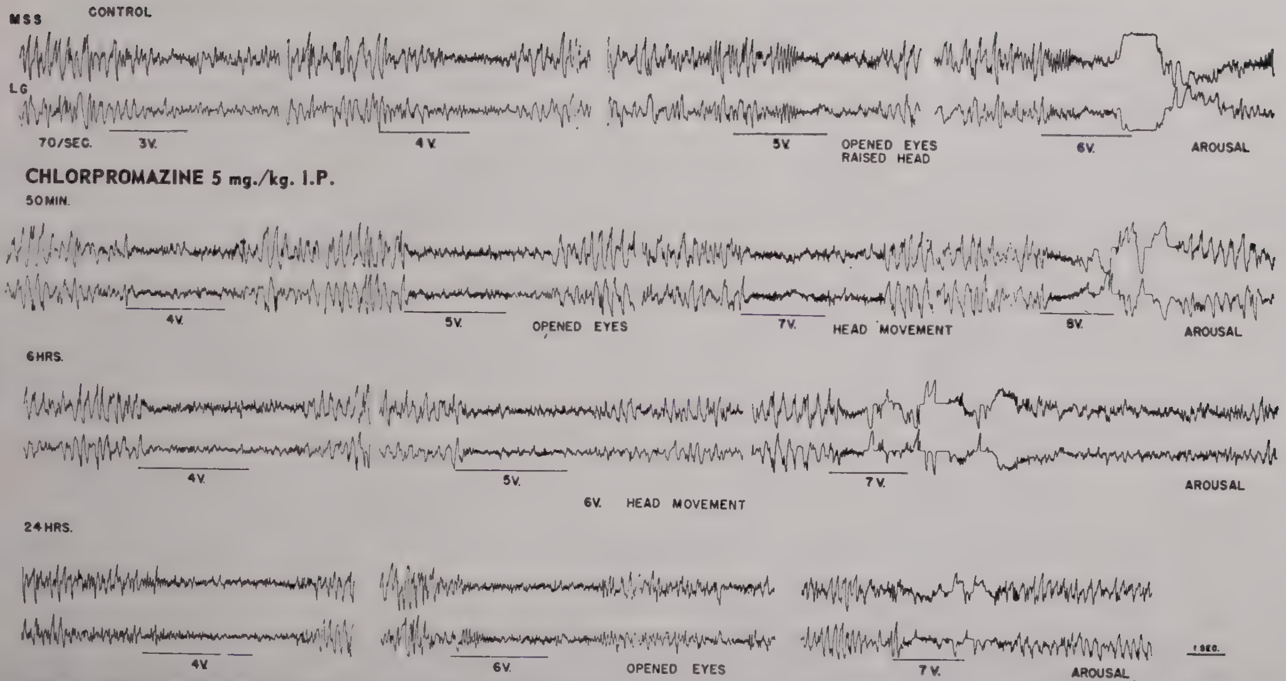

FIGURE 1. Effect of chlorpromazine on electroencephalographic and behavioral changes following stimulation of the midbrain reticular formation. The duration and voltage of the stimulation are indicated by the bars below each pair of tracings. The indicated behavioral response began at the *onset* of stimulation. The leads are from the medial suprasylvian gyrus (MSS) and the lateral gyrus (LG).

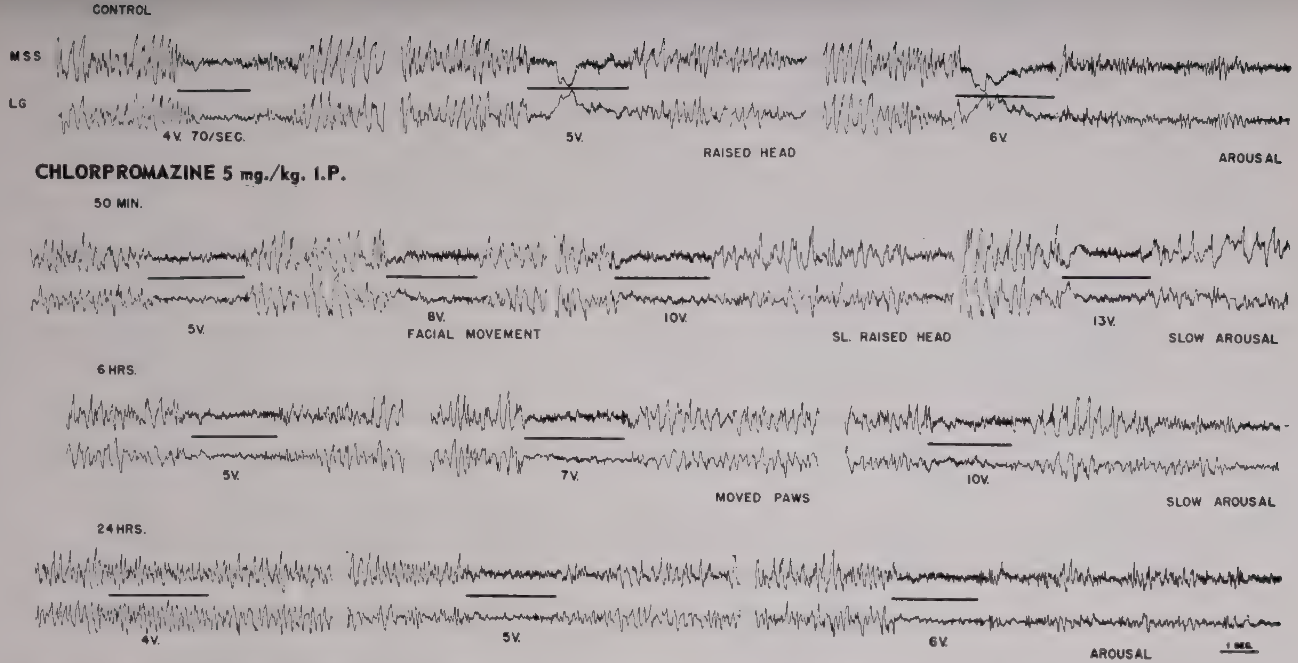

FIGURE 2. Effect of chlorpromazine on electroencephalographic and behavioral changes following stimulation of the diencephalon in the area of the centralis lateralis-center median. The data are from the same experiment as that shown in FIGURE 1. The duration of stimulation is indicated by bars between each pair of tracings. The indicated behavioral response began at the *onset* of stimulation.

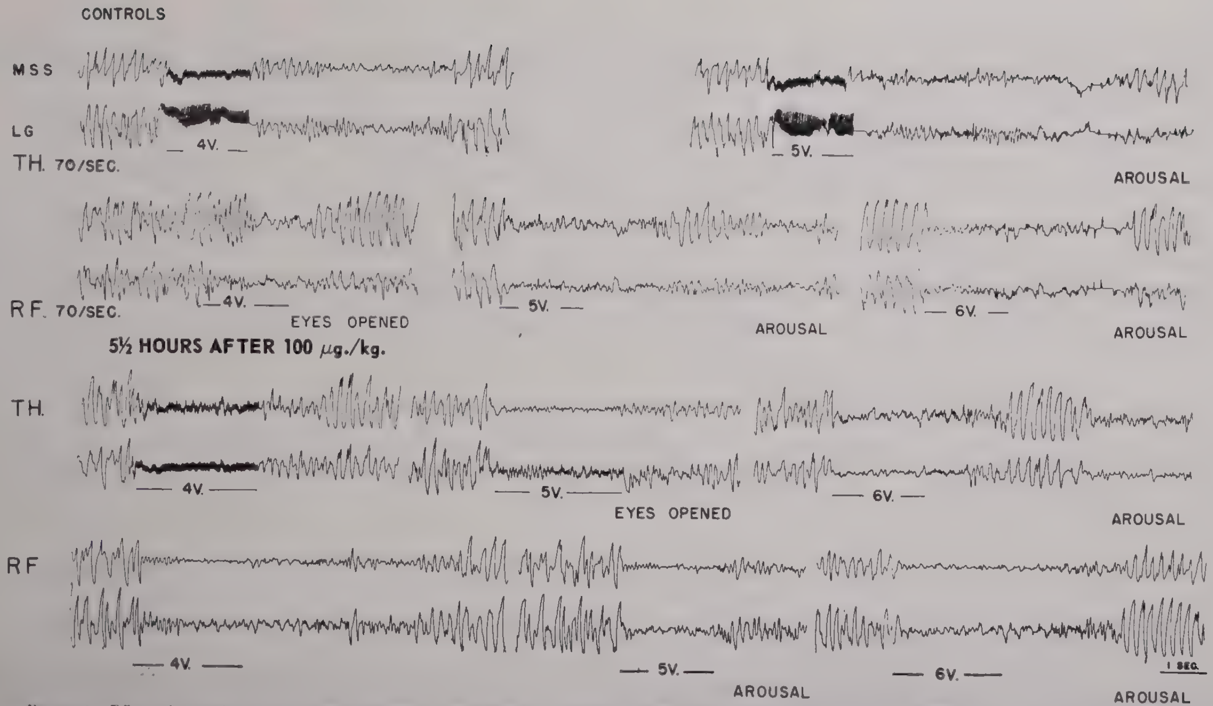

FIGURE 3. Effect of reserpine on electroencephalographic and behavioral changes following stimulation of the diencephalon (TH) and the midbrain reticular formation (RF) in the same cat and with the same electrodes as in FIGURES 1 and 2. The duration and voltage of the stimulation are indicated by the bars below each pair of tracings. began at the *onset* of stimulation.

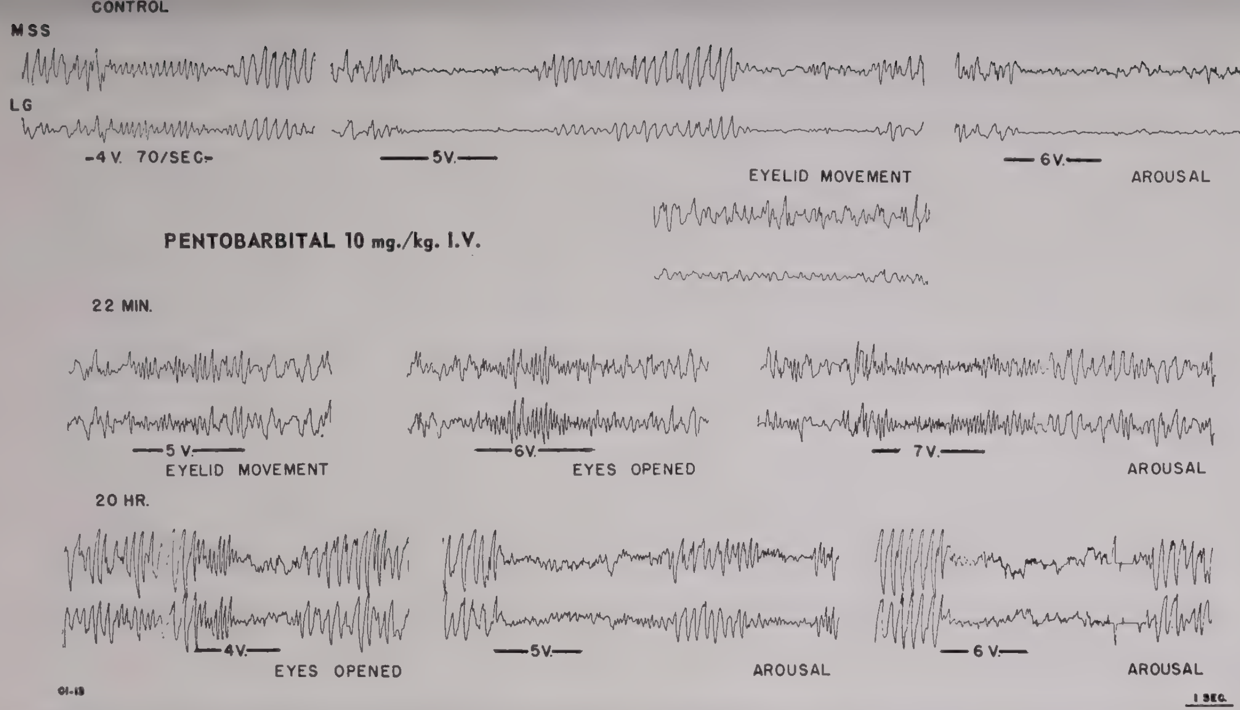

FIGURE 4. Effect of pentobarbital on electroencephalographic and behavioral changes following stimulation of the midbrain reticular formation in the same cat and with the same electrode as in FIGURE 1. The duration of stimulation is indicated by bars below each pair of tracings. Behavioral response began at the *onset* of stimulation.

in the voltage either caused further desynchronization, evidenced by a furthe reduction in amplitude and an increase in frequency (FIGURES 3 and 4), c elicited such a response interrupted by a spindle burst (FIGURE 1). Ther was indication of a brief awakening of the animal. Further increase in th stimulus induced prolonged desynchrony accompanied by an abrupt behaviora arousal from sleep. The behavioral response considered to be "arousal" consisted of a startle response, in which the animal looked about the cage, an body movement occurred immediately after the onset of stimulation, wit wakefulness lasting at least several minutes. This effect following 6 v. stimulation in cat CI 13 is also shown in FIGURES 1, 3, and 4. Forty-seven contro experiments were performed.

Chlorpromazine was administered in 6 of the experiments. Doses of 2 to mg./kg. caused depression and some ataxia. If undisturbed, the animal seemed to prefer to remain immobile, although they could be aroused by peripheral stimulation. EEGs showed patterns of low-voltage fast activity or o high-voltage slow waves, apparently correlated with the state of arousal of th animal. These were not different from those taken on days when no dru was administered. During this depressed period there was only a slight ris in the threshold (1 to 2 v. maximum) at which reticular stimulation cause EEG desynchronization. Typical effects after the administration of chlorpromazine (5 mg./kg.) are shown in FIGURE 1. Although 3 v. stimulatio gave no effect, the desynchronization at 4 v. was almost as great as in the controls. The first signs of behavioral arousal still appeared at 5 v., althoug good, prompt arousal did not occur until an 8 v. stimulus was used. At and 24 hours there was partial recovery to a behavioral arousal at 7 v. Repeated experiments in the same and different animals gave similar results, small but consistent rise in the threshold for EEG activation and behaviora arousal following the administration of chlorpromazine (4 to 6 mg./kg.) These data are in line with those previously reported for acute, curarize preparations (Killam and Killam, 1956b).

Reserpine phosphate, administered to the chronic cats in 12 experiments in doses of 100 μg./kg., caused little if any change in the EEG activation or behavioral arousal responses. Such an experiment on cat CI 13 is shown in FIGURE 3. Following stimulation of the reticular formation (R.F.), arousal occurred at 5 v. both before and after administration of the drug. The EEG desynchronization following 4 v. was, if anything, improved by reserpine, although no consistent change in threshold could be observed in this series of animals. Although slow activity in the EEG and behavioral sleep are evidenced before each stimulus (FIGURE 3), it was our impression that both were more difficult to obtain in cats treated with reserpine. Furthermore, behavioral sleep seemed to be less often accompanied by high-voltage slow waves and spindles than on days when no drug was given. The drug-induced changes in the EEG were far from clear-cut, however.

Low doses of pentobarbital were difficult to study in chronic animals using the intraperitoneal route of administration. So much ataxia occurred at doses of 10 mg./kg. that the animals would not sleep, but moved about very restlessly n an uncoordinated fashion. On slow intravenous administration the animals howed greater depression and were more quiet, but sleep was still difficult to btain. It was therefore not always possible to measure thresholds at the eight of the drug effect. In 8 experiments there was a rise in the threshold for EG and behavioral arousal ranging from 1 to 5 v. FIGURE 4, illustrating he effects of pentobarbital (10 mg./kg.) in cat CI 13, represents the smallest hange found. A fall in amplitude and an increase in frequency in the EEG ccurred at 4 v. in the control and at 7 v. after the administration of pentobarital. At 5 and 6 v. after the drug the only effect of reticular stimulation was n increased frequency of the high-voltage waves. This EEG alteration was ot considered comparable to the normal "arousal response." The threshold or behavioral arousal was raised 1 v., although this arousal was sluggish rather han rapid. The next day thresholds were a little below normal for this cat, nd normal levels returned by afternoon. The effects of pentobarbital on these athways were thus qualitatively similar to those found in curarized animals lthough of less magnitude. As mentioned above, the reduced effectiveness of entobarbital may have resulted from the timing of the threshold determinaions or from the stress induced by the ataxia resulting from the drug.

Effects on EEG activation and behavioral arousal following stimulation of the iffuse thalamic projection system. Parallel studies of the thresholds for EEG nd behavioral changes induced by the stimulation of one of the nuclei of he diffuse thalamic projection system were made each day in each of the anials of this series. FIGURES 2, 3, and 5 show such records taken during the xperiments described in detail above. The effects are similar to those obained in all the animals. In cat CI 13, 4 v. produced threshold EEG changes, nd 5 to 7 v. induced behavioral arousal. The same EEG leads are shown as hose in FIGURES 1 and 4, and the time of each series is within a few minutes of hat of the corresponding series of reticular stimulations.

At 4 to 6 mg./kg., chlorpromazine raised the threshold at which EEG desynhronization could be elicited by 1 to 3 v. The duration of the EEG alteration vas also markedly shortened, and it rarely outlasted the stimulus unless the timulation was more than doubled in voltage. The behavioral effect of halamic stimulation was even more markedly depressed. A typical effect is own in FIGURE 2. Fifty minutes after the administration of chlorpromazine 5 mg./kg.), 4 v. elicited no change in the EEG, and the response to 5 v. as limited to the time of stimulation. At 8 v. the desynchrony still did not utlast the stimulus, and only a slight facial twitch accompanied the EEG hange. At 10 v. the cat raised its head briefly, while the first evidence of rue behavioral arousal from sleep did not occur until 13 v. were applied to the halamic site. Up to 15 v. the awakening was sluggish and short-lasting. Six ours after drug administration there was a partial recovery, and by 24 hours oth the EEG and behavioral responses had returned almost to normal levels. his separation of the EEG and behavioral response to thalamic stimulation ppeared in all the experiments with 4- to 6-mg./kg. doses of chlorpromazine nd thus appears to be of considerable importance in the action of the drug.

In the acute experiments reported previously, using data from EEG tracings

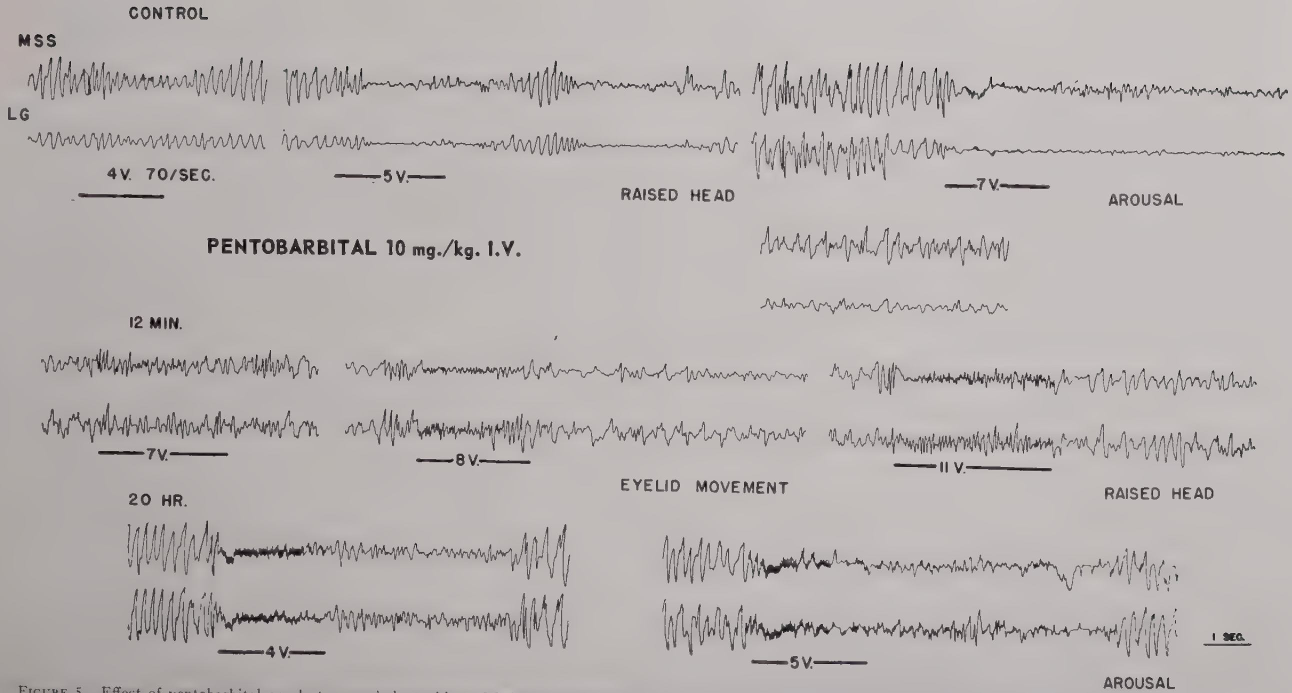

FIGURE 5. Effect of pentobarbital on electroencephalographic and behavioral changes following stimulation of the diencephalon in the same cat and with the same electrode as in FIGURE 2. The duration and voltage of stimulation are indicated by the bars below each pair of tracings. Behavioral response began at the *onset* of stimulation.

lone, chlorpromazine had less effect on arousal from this site than on that from eticular formation. It is not clear whether this is wholly because thresholds or EEG change are not a sufficiently sensitive measure of drug effect or whether ther factors such as tubocurarine, artificial respiration, support of body tem- erature, and the like are of importance.

Reserpine had only slight effects on EEG and behavioral arousal following ne stimulation of the diffuse thalamic projection system. As shown in FIGURE , a dosage of 100 μg./kg. only slightly shortened the duration of the EEG esynchronization and decreased the promptness and magnitude of behavioral rousal rather than altering its threshold. Changes in this and other experi- nents were not considered significant.

Pentobarbital raised the threshold at which EEG desynchronization could e induced by 3 to 5 v. Behavioral arousal was even more affected. Typical ffects are shown in FIGURE 5 for cat CI 13. The EEG desynchronization, ppearing at 5 v. and outlasting the stimulus by a minute or more at 7 v. in the ontrols, was elicited only at 8 v. following the administration of pentobarbital 10 mg./kg.), and even following an 11-v. stimulation of the thalamus the EEG showed neither as low an amplitude nor as long-lasting an effect as those videnced in the controls. Behavioral arousal, evidenced in the control at 7 v., vas sluggish and short-lasting after the pentobarbital even following the 11 v. timulus. The next day control thresholds were below normal and recovered y afternoon.

The depression evidenced in these experiments following the administration of entobarbital is in contrast to the enhancement of the recruiting potentials ob- ained in curarized animals by the slow stimulation of the same nuclei of the iffuse thalamic projection system (Domino, 1955; King, 1954, 1956). Re- ruiting was therefore studied in the chronic cats of this series. The threshold nd amplitude of the recruiting response obtained by thalamic stimulation at /second were very variable in waking animals. Both varied so markedly vith ambient noise level, sleep patterns, and the like that it was not possible o obtain reliable data as to the drug effects on these potentials.

Effects on seizure thresholds in the limbic system. The thresholds at which hinencephalic seizures could be elicited were determined in cats having elec- rodes chronically implanted in the hippocampus, amygdala, and cortex, as well s in some of the following sites: centralis lateralis, center median, or ventralis nterior of the thalamus, reticular formation of the midbrain, and entorhinal ortex. For these determinations the amygdala was stimulated in 2 cats, and ne hippocampus was stimulated also in 2 cats. The stimuli were delivered at 50/second for 3½ seconds at a subthreshold voltage. Every 10 seconds the timulus was repeated with increased voltage until a seizure was elicited in hinencephalic leads. The time duration of the rhinencephalic seizure (*R* in IGURES 6 and 8) was measured as that time during which high-voltage convul- ve activity in the EEG was confined to the rhinencephalic leads, and when here was no manifestation of clonic or tonic convulsive motor activity in the nimal. During this period the animal showed cessation of movement, staring, cking, and occasionally salivation. If the seizure spread to include the corti- al areas, there were spasmodic jerks of the face and neck musculature or severe

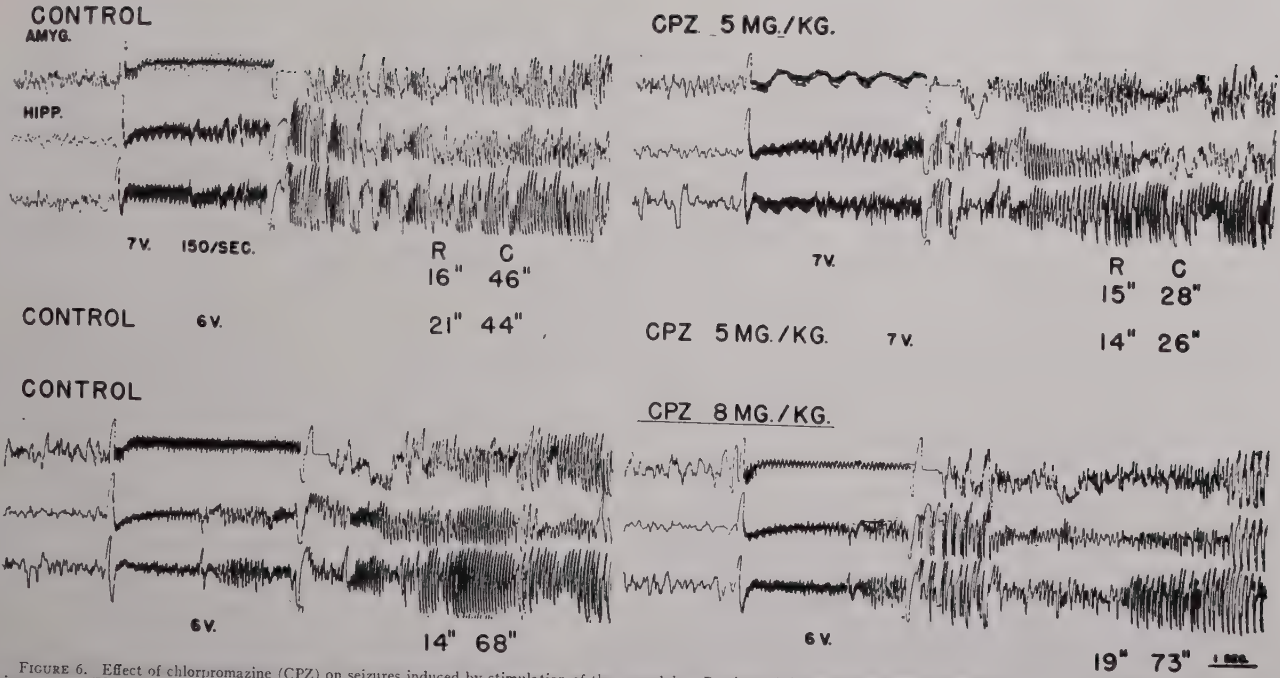

FIGURE 6. Effect of chlorpromazine (CPZ) on seizures induced by stimulation of the amygdala. Portions of the recordings from the ipsilateral amygdala (AMYG.) and ipsilateral hippocampus (HIPP.) are shown, with durations of the rhinencephalic (R) and clonic phases (C) indicated in seconds.

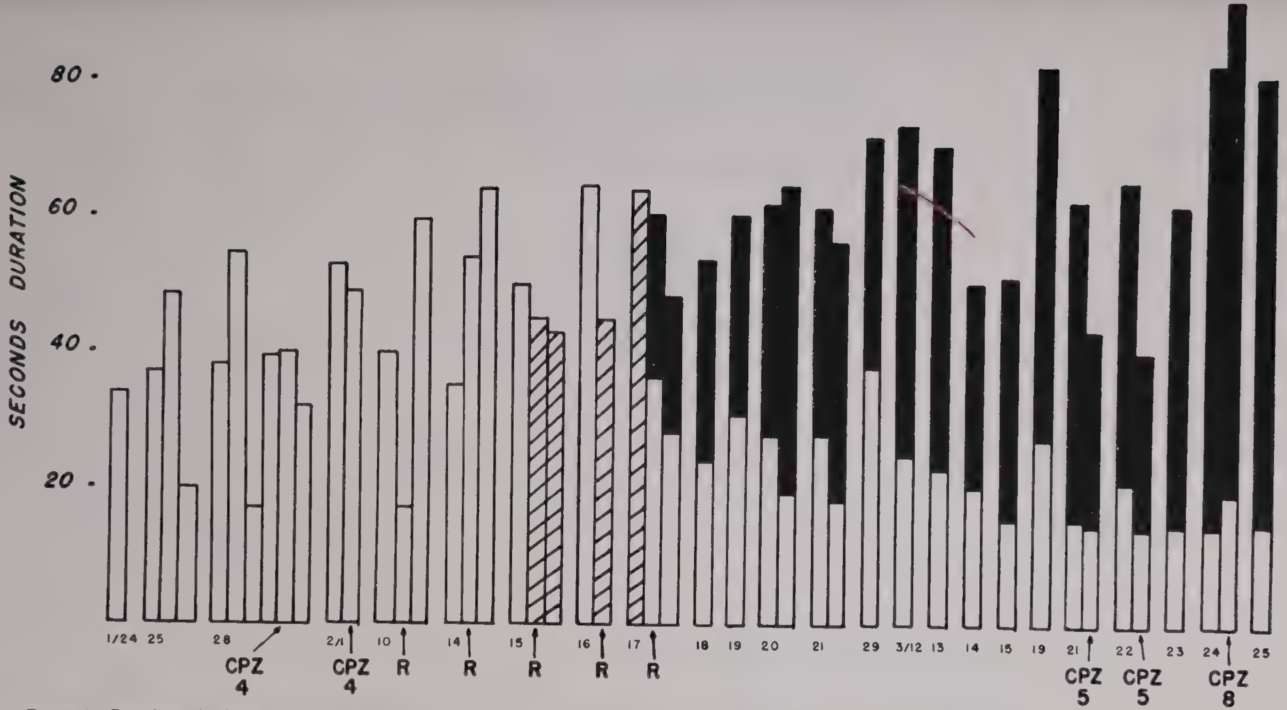

FIGURE 7. Durations of seizures following amygdala stimulation. The clear bars indicate rhinencephalic seizures; crosshatched bars, rhinencephalic seizures accompanied by muscular spasms and jerks of the face and neck; solid black portions of bars, the time in which generalized seizures with severe clonic components occurred. The figures below each group of bars show the date of the experiment. Drugs were administered before the seizure indicated by arrow—chlorpromazine (CPZ) 30 minutes and reserpine (R) 3 hours before the test seizure.

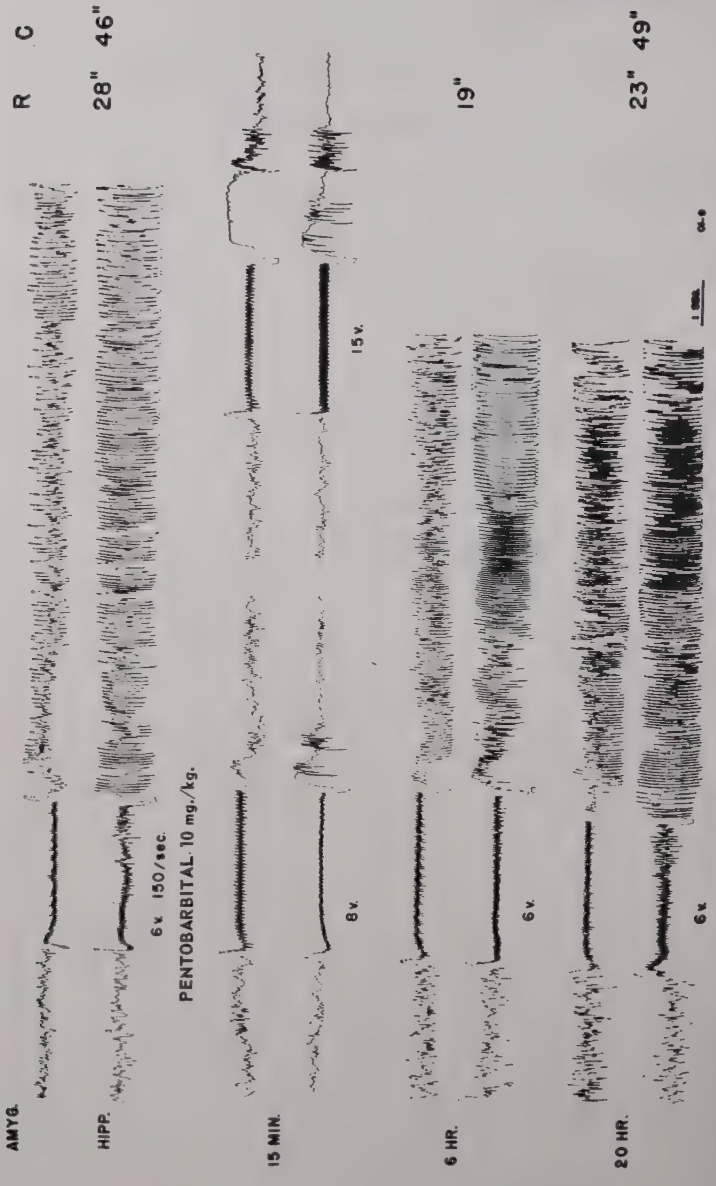

FIGURE 8. Effect of pentobarbital on seizures elicited by stimulation of the amygdala. Ipsilateral amygdala (AMYG.) and hippocampal (HIPP.) tracings during and following stimulation are shown. The durations of the rhinencephalic (R) and clonic phases (C) are given in seconds.

clonic convulsive activity of the entire body. Such portions of the seizures were recorded as clonic (*C* in FIGURES 6 and 8).

Seizure thresholds measured in 38 control experiments showed surprising stability under these conditions if stimulation of the rhinencephalon was not attempted sooner than 3 hours after any seizure activity had been elicited. At shorter time intervals of stimulation there was a tendency for thresholds to rise markedly. In the animals studied for periods of 2 to 7 months, the greatest variability in control seizure thresholds under these conditions was 1 v. Changes of this magnitude following drug administration were therefore not considered significant.

Chlorpromazine was administered in 7 of the experiments. When there were no motor evidences of generalized seizures, and when EEG convulsive activity did not spread to the isocortex, the drug had little effect on the threshold or duration of the seizures elicited by direct stimulation of the limbic system. This effect on days 1/28 and 2/1 is shown in FIGURE 7. When stimulation of either the amygdala or hippocampus evoked seizures involving the cortical as well as the rhinencephalic structures, however, motor convulsive patterns were evidenced, and chlorpromazine markedly shortened this clonic component of the convulsion. Portions of the convulsive EEG tracings from 3 such experiments are shown in FIGURE 6. Although the administration of chlorpromazine (5 mg./kg.) neither altered the threshold at which seizure activity was induced nor changed the pattern or duration of the rhinencephalic component (*R*) significantly, the clonic portion of the seizure was reduced about one third, from 46 to 28 seconds. Rhinencephalic high-voltage activity ceased simultaneously with that in the isocortex. Twenty-four hours later the duration of the clonic component had returned to the normal range. A second dose of chlorpromazine again reduced the clonic component significantly, from 44 to 26 seconds, while the rhinencephalic phase was reduced only slightly. Two days later a larger dose of chlorpromazine (8 mg./kg.) was given that appeared to prolong slightly both phases of the seizure.

Single doses of 100 μg./kg. of reserpine phosphate failed to cause significant alterations in the threshold or duration of seizures induced by stimulation of the amygdala or hippocampus. In line with the clinical suggestions that the chronic administration of reserpine may cause increased seizures in susceptible patients, we therefore undertook the study of repeated doses in chronic cats. FIGURE 7 shows the seizures elicited in the last 3 months of a 7-month testing period for cat CI 9. For the first 4 months, stimulation of the amygdala elicited only rhinencephalic seizure activity accompanied by salivation, chewing, and the like. A few control days at the beginning of the fifth month are illustrated. The threshold voltage for 7 of the 10 controls taken between days 1/24 and 2/10 was 6 v. The seizure durations illustrated ranged from 17 to 54 seconds, with an average of 38 seconds. A single dose of reserpine at 100 μg./kg. prolonged the seizure slightly on that day to 49 seconds. Four days later a seizure was again elicited, and its duration was approximately that of the average control (35 seconds). Again a single dose of reserpine prolonged the seizures to an average of 59 seconds. The following day a second dose of reserpine was given. Stimulation of the amygdala 3 and 6 hours after administration of the drug elicited rhinencephalic seizures accompanied by neck and facial twitching, as shown by the crosshatched bars. The following day (2/16) the control seizure was similar to that of earlier controls, with no motor manifestations of spread in the seizure activity. Following the administration of reserpine the seizure following amygdala stimulation was accompanied by twitching of considerable severity. On the fourth successive day of this series the control seizure showed similar motor components. After the administration of reserpine, stimulation of the amygdala was followed by a seizure confined at first to the rhinencephalon and then progressing into a full pattern of clonic convulsive activity, as indicated by the black bar. Although no further reserpine was given to this cat, stimulation of the amygdala elicited this pattern of rhinencephalic and clonic seizures in all experiments thereafter.

A similar chronic series was undertaken in an animal showing rhinencephalic seizures following stimulation of the hippocampus. The average control seizure over a month's period was 15 seconds in duration. Over a period of 5 days of daily administration of reserpine the average seizure duration following drug administration was increased to 46.5 seconds. Motor manifestations accompanied the seizure elicited following the third dose of reserpine and also appeared on the fourth and fifth days. The drug was discontinued after 5 doses, and the seizure pattern reverted to the simple rhinencephalic type after 2 additional days. Comparative data for these 2 chronic series are given in TABLE 1.

Pentobarbital in 5- to 10-mg./kg. doses exerts remarkable blocking activity in these preparations, as noted by Green and his collaborators (1956) in acute experiments. In 6 experiments, seizures confined to the rhinencephalon as well as those having clonic components and isocortical seizure patterns were both prevented by pentobarbital, even when the voltage of stimulation was raised to 3 times the control threshold. An example of this effect in the same cat as shown in FIGURES 6 and 7 is shown in FIGURE 8. Fifteen minutes after a dose of pentobarbital (10 mg./kg.) was administered, no seizure could be elicited following stimulation of the amygdala. Six hours later, when the depressant effects of pentobarbital had apparently disappeared, stimulation of the amygdala elicited a short seizure confined to the amygdala and hippocampus

TABLE 1

EFFECT OF RESERPINE ADMINISTRATION ON SEIZURE DURATION

	No. observations	Average threshold	Seizure duration Seconds	$S\bar{x}$
Amygdala				
Control	10	6	38	3.9
During chronic reserpine administration (5 doses)	11	6	53	2.4
Hippocampus				
Control	9	5	15	1.4
During chronic reserpine administration (5 doses)	9	4	46	3.0

with no cortical or clonic components. By 20 hours a typical combined seizure similar to that seen in the control was elicited. Similarly, when seizures were elicited by stimulation of the hippocampus, pentobarbital prevented the appearance of electrical convulsive patterns and of muscular twitching. An example of this effect is shown in FIGURE 9. Recovery is shown in this animal at 20 hours.

Discussion

EEG and behavioral arousal. In chronic experiments relating EEG and behavioral arousal, selective drug effects have been demonstrated that were not observable in similar experiments on curarized cats. The most striking change noted following pharmacological doses of chlorpromazine was the elevation of the threshold for EEG activation and behavioral arousal elicited from the diffuse thalamic projection system. The effect is in contrast to the relatively small change in the threshold for EEG activation obtained in curarized cats (Killam and Killam, 1956a, 1956b). These data, however, correlate with the findings that doses above 1 mg./kg. in the curarized cat depress the recruiting potentials elicited from these nuclei (Killam and Killam, 1956a), and with the finding that the spindle activity in the *cerveau isolé* is depressed by chlorpromazine at still lower doses in the noncurarized animal (Das *et al.*, 1955).

The threshold and duration of EEG and behavioral arousal following stimulation of the midbrain reticular formation were only slightly altered by the same dose of chlorpromazine that markedly depressed thalamocortical circuits. Thresholds for EEG activation were consistently increased by approximately 1 v., and the duration of the response was somewhat reduced, just as was previously reported for the curarized cat (Killam and Killam, 1956a). Thresholds of behavioral arousal paralleled those of electroencephalographic changes. These data agree with those of Preston (1956b) and Terzian (1954), who found the chlorpromazine-induced changes in electrocortical activation patterns in curarized cats to be unimpressive. Somewhat greater depression of EEG activation following reticular stimulation was reported by Hiebel *et al.* (1954) for cats with prebulbar section. It is possible that such preparations were sensitized to the drug, as suggested by Das *et al.* (1954). In curarized rabbits, on the other hand, Rinaldi and Himwich (Himwich, 1956; Himwich and Rinaldi, 1955–56; Rinaldi and Himwich, 1955b) have demonstrated a shortening of the EEG arousal response and a marked increase in the threshold at which it may be obtained by stimulation of the midbrain reticular formation or by peripheral stimuli. Similar effects have been noted on EEG responses to hypothalamic stimulation in curarized rabbits (Longo *et al.*, 1954) and rats (Barraclough, 1956). These responses may represent a differential sensitivity of rodents.

Reserpine in doses of 100 μg./kg. failed to alter electroencephalographic and behavioral changes induced by stimulation of either thalamic or reticular sites. These data confirm the EEG findings in similar experiments on curarized cats (Killam and Killam, 1956a, 1956b). In rabbits, Rinaldi and Himwich (1955a) have shown that doses of reserpine up to 500 μg./kg. had little effect on arousal responses. At doses of 1 mg./kg. or more, however, the EEG alerting patterns were more frequent, and the threshold at which reticular

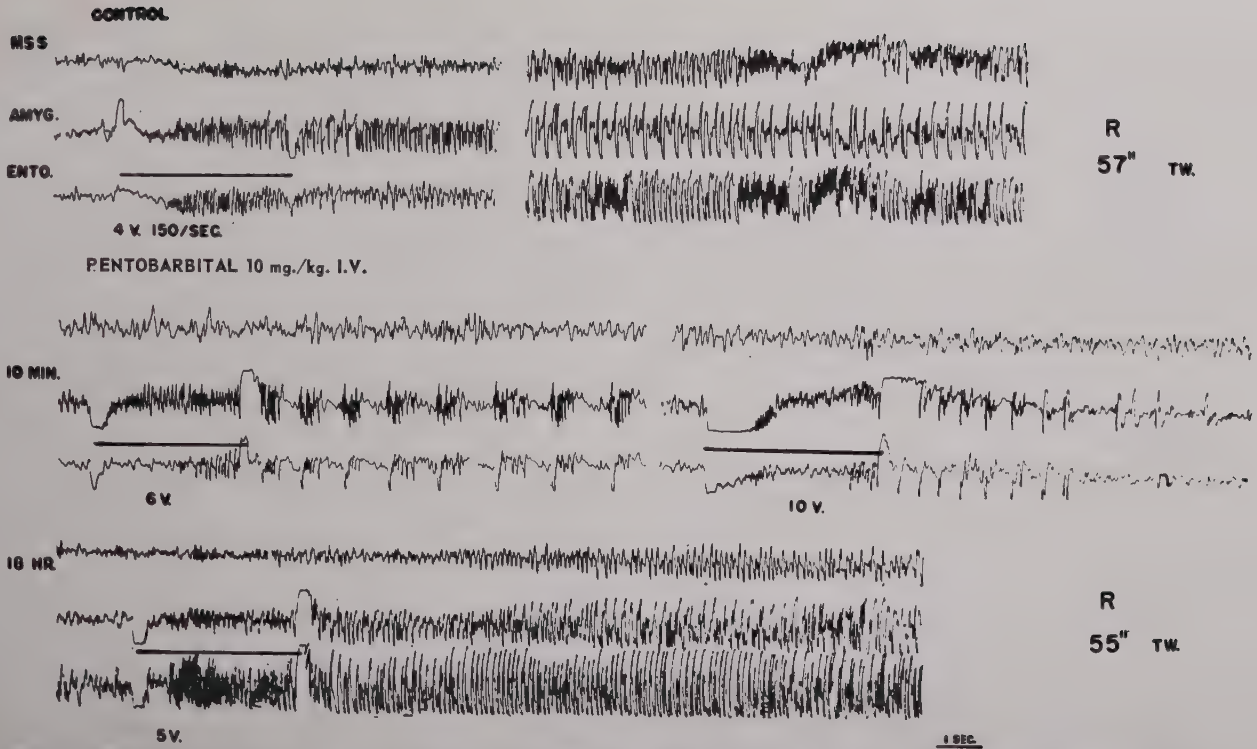

FIGURE 9. Effect of pentobarbitol on seizures elicited by stimulation of the hippocampus. Ipsilateral median suprasylvian (MSS), amygdala (AMYG.), and entorhinal tracings during and following stimulation are shown. The durations of the rhinencephalic seizures accompanied by muscular twitching are shown in seconds.

stimulation evoked EEG changes was progressively reduced. These authors consider the behavioral depression to result from hypothalamic inhibition. Bein (1953) suggests the possibility that reserpine activates a rostral inhibitory mechanism. Barraclough (1955), on the other hand, suggests that there may be inhibition of the bulbar reticular formation of the rat. Inhibition of the evoked responses and afterdischarges elicited from reticular, thalamic, and cortical sites in the rabbit have also been reported (Gangloff and Monnier, 1955) to follow the administration of 1.5 mg./kg. of reserpine.

Pentobarbital depressed the EEG activation and behavioral arousal elicited from diencephalic and reticular sites. Doses causing comparable depression of general behavior were slightly more effective than chlorpromazine in elevating the threshold for EEG changes following the stimulation of either site. The threshold for behavioral arousal was not, however, increased by pentobarbital as much as by chlorpromazine, and there seemed less evidence of selective action on the thalamocortical circuits. The depression of the EEG and behavioral changes elicited by the stimulation of the reticular formation confirms the evidence reported earlier (Arduini and Arduini, 1954; French *et al.*, 1953; Domino, 1955; King, 1954, 1956). Although low doses of pentobarbital had been shown to enhance the recruiting responses (King, 1954, 1956; Domino, 1955; Jasper *et al.*, 1955) elicited from diffusely projecting nuclei of the thalamus, it is not surprising that the response to high-frequency stimulation of this area was depressed by pentobarbital (10 mg./kg.). The optimal frequency of stimulation for recruiting responses has been shown to be reduced by the compound (Domino, 1955), and there is evidence of a prolonged neuronal recovery time in relay nuclei (Marshall, 1941; King *et al.*, 1955), and in brainstem reticular formation (King *et al.*, 1955). Similar delays in recovery of neurons in thalamocortical circuits could make them unresponsive to rapid stimulation.

EEG patterns. There have been numerous reports of increased slow-wave and spindle activity in the EEGs of patients (Terzian, 1952), monkeys (Das *et al.*, 1954), cats (Hiebel *et al.*, 1954), and rabbits (Terzian, 1954; Longo *et al.*, 1954; Rinaldi and Himwich, 1955b) given moderate doses of chlorpromazine. In noncurarized, nonanesthetized cats allowed to set their own sleep-wakefulness cycles, chlorpromazine did not induce or enhance such EEG patterns or increase the incidence of normal sleep.

Some evidence of an increase in the average frequency of the EEG and a reduction in the 10 to 14/second spindle activity was apparent in cats treated with reserpine. The changes were not dramatic, however, and the drug could not be considered directly to induce EEG activation patterns in the cat. No significant alterations in the EEG or in behavioral sleep have been observed in noncurarized monkeys (Monroe *et al.*, 1955; Schneider and Earl, 1954). In contrast, marked electroencephalographic changes have been reported to follow the administration of large doses of reserpine to the curarized rabbit (Rinaldi and Himwich, 1955a, 1955b; Himwich, 1956). Two and one-half mg./kg. doses and above elicited long-lasting low-voltage fast activity. In the cat, doses of 1 mg./kg. (about 20 times the dose showing miosis, diarrhea, and depression in the series reported here) have been reported to cause long-lasting, desynchronized, low-voltage cortical activity accompanied by synchronous waves in the limbic structures (MacLean *et al.*, 1955–56).

Limbic system. The effect of chlorpromazine in shortening the generalized seizure phase of convulsive activity induced by stimulation of the hippocampus or amygdala has not previously been reported. Early findings that the compound showed anticonvulsant activity (Courvoisier *et al.*, 1953) were not confirmed by standard anticonvulsant testing procedures. In low doses, chlorpromazine neither raised the threshold nor shortened the duration of seizures confined to the limbic area. In contrast, its effect on rhinencephalic seizures induced by the stimulation of inflow pathways of the fornix was clearly depressant in curarized animals (Killam and Killam, 1956b). The slight increase in seizure duration following chlorpromazine (8 mg./kg.) may have had some significance in view of Preston's (1956a, 1956b) findings that toxic doses (40 mg./kg.) induced spiking activity in amygdaloid nuclei.

Reserpine, however, while unimpressive in single doses, clearly enhanced rhinencephalic seizure activity when several successive daily doses were administered. Seizures confined to limbic areas showed a spread to cortical regions accompanied by motor manifestations of convulsive activity, and such changes appeared irreversible. These results are in agreement with data from curarized cats (Killam and Killam, 1956b) given reserpine, in which several cases of spontaneous rhinencephalic seizures were reported, and induced seizures in this system were prolonged. Other evidence of reduced seizure threshold (Chen *et al.*, 1954; Jenney, 1954) and facilitation of seizure spread (Chen and Ensor, 1954) has been obtained in mice treated with the drug.

Pentobarbital was clearly the most effective blocking agent for seizures induced by stimulation of limbic structures. Similar data had been reported previously for curarized cats (Killam and Killam, 1956b) and rabbits (Green, 1956).

General considerations. On the basis of the evidence presented, the mechanism of action of tranquilizing agents cannot be considered to be the selective depression of the reticular activating system. The principal actions of chlorpromazine are more probably anterior in some arousal systems in which the diffuse thalamic projection represents a major link. One might speculate that such a system also involves outflow circuits from the rhinencephalon, since chlorpromazine reduced seizures that had become generalized rather than those confined to limbic structures. Other data for this hypothesis are suggested by the blockade of arousal responses in the amygdala and hippocampus (Killam and Killam, 1956b). Reserpine appears to act by some other mechanism. Pathways for EEG activation and behavioral arousal were not affected by the drug while seizure spread within the limbic system or its outflow was facilitated. The data from these investigations do not yet provide information as to whether the pathways on which the effects of reserpine and chlorpromazine have been demonstrated are related to the sedating actions of the compounds or to their "psychotherapeutic" effects, or perhaps to both.

The pharmacological data presented have important implications with regard to concepts of the electrical manifestations of sleep and wakefulness. In general agreement with current concepts (French *et al.*, 1952; Lindsley *et al.*, 1949; Lindsley *et al.*, 1950; Magoun, 1950a, 1950b, 1952; Moruzzi and Magoun, 1949), long-lasting low-voltage fast activity usually appeared to be associated with behavioral wakefulness or alertness under control conditions. The parallelism was not complete, however, especially when the brainstem was stimulated with merely threshold voltage. A short desynchronization, usually not outlasting the stimulus by more than a few seconds, could be induced with a slightly lower voltage of stimulation than could behavioral arousal. Under the influence of chlorpromazine, however, behavioral arousal was clearly dissociated from EEG desynchronization, especially when the responses were elicited by stimulation in the diencephalon. Thresholds for EEG response were raised much less than thresholds for behavioral arousal. Further evidence of the separability of EEG desynchronization and behavioral arousal was noted in cats given reserpine. Behavioral evidences of sleep were often accompanied by EEG records showing a predominance of low-voltage fast activity. Other evidence of the dissociation of EEG desynchronization and behavioral arousal by pharmacological agents includes that of Wikler (1952), using atropine in the dog, and Longo (1956), studying the effects of atropine in the rabbit. It is clear that the various pathways directly involved in, and exerting tonic influence upon, the EEG activation pattern and behavioral arousal must be reinvestigated. Pharmacological agents will be an important tool for such investigations.

Summary

In cats with chronically implanted electrodes, the effects of reserpine, chlorpromazine, and pentobarbital have been studied on EEG and behavioral responses elicited by brain-stem or rhinencephalic stimulation. Chlorpromazine markedly influenced the EEG desynchronization and behavioral arousal responses elicited by rapid stimulation of the diencephalon, as did pentobarbital. The effect of chlorpromazine on similar responses elicited by stimulation of the reticular formation was slight, with a small but consistent depression being noted following doses causing marked behavioral depression. Reserpine did not influence these pathways. Seizures induced by direct stimulation of the amygdala and hippocampus were facilitated, and their spread to the isocortex with accompanying motor manifestations was enhanced by reserpine. The duration of motor components of such seizures was reduced by chlorpromazine. Pentobarbital exerted marked blocking actions on the rhinencephalic and generalized seizures. The dissociation of EEG desynchronization and behavioral arousal under the influence of "psychotherapeutic" compounds has been pointed out, and its implications in general concepts of sleep and wakefulness have been discussed.

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THE BIOCHEMICAL EFFECTS OF PSYCHOTHERAPEUTIC DRUGS[*]

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As of this writing, the following tranquilizing drugs have been brought to the attention of the biochemist: reserpine, the derivatives of phenothiazine and, in particular, chlorpromazine, azacyclonol (Frenquel), and meprobamate (Miltown). This situation, of course, changes from day to day, since additional drugs are added continuously to this group. It is probably safe to say that, in a summary with the above title 5 years from now, most of the drugs mentioned will not be discussed any longer, and that new ones will have taken their places. It is an obvious desire of the biochemist to deal with a less ephemeral subject than the biochemical mechanisms underlying the action of a particular drug that probably will be superseded within a short time.

It should be remembered, furthermore, that drugs with apparently similar action that have been lumped together as tranquilizing drugs exhibit a variety of chemical structures such as the substituted aliphatic propandiol (Miltown), and substituted phenothiazine (chlorpromazine), and the ester of trimethoxybenzoic acid with a complex heterocylic structure containing the indole ring. It may be expected that the biochemical mechanisms underlying the action of these drugs might, in one case, be due to influences on membranes and permeability; in another, primarily to effects on enzymatic processes related directly to function; and, in still another case, to effects on enzymatic mechanisms far removed from the functional system. One might also consider that the action of some of these drugs may not relate specifically to any particular metabolic mechanism at all, but that their solubility and spatial configuration results in their attachment to structures in the cell, which causes interference with a multiplicity of biochemical mechanisms. The biochemist will attempt to extract from the recently accumulated knowledge of the biology of these drugs the aspects of more general metabolic significance, and he will be interested in those findings that indicate the properties of the cells in their reactions toward these drugs.

From this point of view, the study of chlorpromazine was taken up some time ago,[1, 2] and it is on the basis of the results obtained in this laboratory that biochemical studies related to substituted phenothiazine will be reviewed. Little if any biochemical research has been done on the other tranquilizing drugs. The main interest in reserpine at present is on a pharmacological level as discussed in this monograph. Recently C^{14} reserpine has been administered to rats, and the metabolism of the drug has been studied in whole animals and in tissue slices. The liberation of trimethoxybenzoic acid and the cleavage of the 4-methoxy group (which was labeled) were demonstrated.[3] When the distribution of C^{14} reserpine in the brain was determined, no preferred accumulation of the label was found in any of the functional areas that were analyzed.[4]

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In the development of chemotherapy, one phenothiazine, that is, methylene blue, has played a significant role. This dye may be called one of the mother substances of a large number of chemotherapeutic drugs. It acts as a hydrogen acceptor in enzymatic reactions, it has been used as a vital stain, and P. Ehrlich[5] has noted that it had some curative effect as an antimalarial agent. It also acts as an inhibitor of cholinesterase, a property that it shares with chlorpromazine.[6]

The action of substituted and unsubstituted phenothiazines has been studied in a limited number of investigations on the enzymatic level, on the level of cell organelles, with tissue slices, and in the whole animal. In a search for the mechanism of the anthelmintic action of phenothiazines, Collier and Allenby[7, 8] found that phenothiazine is a strong inhibitor of the over-all oxidation of succinate, and of hexokinase, and of cytochrome oxidase. The same is true for the sulfoxide of phenothiazine and for phenothiazone. It was also found that the oxidation of succinate by liver mitochondrial preparations was inhibited strongly by these compounds. In these experiments very small amounts of mitochondrial preparations corresponding to 0.1 mg. of protein nitrogen per experiment were used. The enzyme inhibitions found were not considered to be responsible for the anthelmintic action of the phenothiazine, and the possibility was discussed as to whether or not semiquinones formed secondarily might be the actual inhibitors by virtue of their interaction with sulfhydril groups.

There is no reason to assume that the effect of unsubstituted phenothiazines, of phenothiazone, and of the sulfoxide are related to that of the substituted phenothiazine, such as chlorpromazine, in which solubility, as well as charge, is changed decisively by the side chain and by ring substitution. The distribution of chlorpromazine labeled with radioactive sulfur (S^{35}) after intraperitoneal injection in rats has been studied by Wase, Christensen, and Polley.[9] Amounts of chlorpromazine corresponding to a daily dose of 3.5 gm. in man were injected daily into groups of 6 animals that were analyzed on 4 successive days. S^{35} was found in all parts of the brain (TABLE 1). It is not easy at present to interpret the results. There is a twelvefold jump of activity in the cortex of the brain from the first to the second day, with only a doubling of activity in

TABLE 1*

ACCUMULATION OF S^{35} CHLORPROMAZINE IN LIPID FRACTION OF BRAIN†

Days	1	2	3	4
Cortex	.08	1	34	31
Pons, medulla, mesencephalon	15	21	31	31
Hypothalamus	21	51	63	100
Thalamus	31	32	14	17
Cerebellum	9	28	22	19
Liver	17	29	23	25

* Adapted from Wase, Christensen, and Polley.
† Counts in thousands.

the liver. There are other features of the results that are not clear, such as the large drop of activity in the thalamus from the second to the third day. A present, the interpretation by the authors must be accepted with caution namely, that chlorpromazine accumulates preferentially in the hypothalamus a suggestion that, from the point of mechanism, has undoubtedly intriguing possibilities, and that may find support in the observations of Grenell, Mendelson, and McElroy.[10] It may be recalled that no preferential accumulation in any functional brain area was observed in the study in which the distribution of C^{14} reserpine in the brain was investigated.

Grenell, Mendelson, and McElroy[10] administered chlorpromazine to rats in amounts of 10 or 50 mg./kg. per day, and they found a significant increase in the concentration of adenosine triphosphate (ATP) in the brain, particularly in the thalamic-hypothalamic region. These authors suggest that the accumulation of ATP may be the result of the inactivity of the animals, which leads to a decreased utilization of the nucleotide. A similar explanation has been suggested for the hyperglycemic effect of chlorpromazine in the mouse and the hamster, an effect that could not be shown in the rat.[11] While the reports discussed above deal with the fate and the action of chlorpromazine in the whole animal, there are a number of observations that concern the effect of chlorpromazine on the metabolism of certain cell organelles, that is, the mitochondria.

We have studied in some detail the effect of chlorpromazine on oxidative phosphorylation in the mitochondria of the liver and the brain [1, 2] (TABLE 2). Chlorpromazine uncoupled oxidative phosphorylation quite effectively in rat-liver mitochondria in a concentration of 2×10^{-4} M. This was true for all substrates used, such as α-ketoglutarate, succinate, pyruvate + malate, and glutamate. Although the effect of the drug on respiration was slight, if present at all, the yield of ATP, as measured with the hexokinase glucose system as trap, was considerably lower in the presence of the drug. It was at first a

TABLE 2

EFFECT OF CHLORPROMAZINE (CPZ) ON OXIDATIVE PHOSPHORYLATION OF MITOCHONDRIA FROM THE LIVER AND FROM THE BRAIN CORTEX OF THE RAT

	Liver			Brain		
	ΔP μmol	O μatom	P:O	ΔP μmol	O μatom	P:O
α-Ketoglutarate	29.3	10.4	2.8	7.9	2.6	3.0
+ CPZ	21.9	11.6	1.9	6.6	2.2	3.0
Succinate	24.9	11.3	2.2	8.2	5.0	1.6
+ CPZ	12.1	10.5	1.1	7.1	4.6	1.5
Pyruvate + malate	20.7	8.2	2.5	14.7	5.5	2.7
+ CPZ	13.2	7.5	1.7	15.1	5.5	2.8
Glutamate	31.3	13.4	2.3			
+ CPZ	19.8	11.6	1.7			

The reaction vessels contained (in μmol): phosphate buffer, pH 7.4, 60; magnesium sulphate, 20; adenosine triphosphate, 6.5; cytochrome c, 0.02; sodium fluoride, 10 to 15; substrate; mitochondrial suspension, 0.5 ml. (average N = 1.3 mg.); glucose, 50; hexokinase (Pabst), 0.4 mg.; and potassium chloride solution (0.15 M) to a final volume of 3 ml. (when brain mitochondria were used, diphosphopyridine nucleotide at 0.2 mg. per flask was added). Levels of substrates: α-ketoglutarate, 30; succinate, 30; glutamate, 60; pyruvate, 60 + malate, 3. CPZ = 2×10^{-4} M. Temperature 37° C. for liver, 30° C. for brain; incubation, 20 to 30 minutes in air.

considerable surprise, not devoid of disappointment, when it was found that chlorpromazine in the same concentrations did not affect the oxidative phosphorylation of mitochondria from the brain cortex. The P/O (phosphorus/oxygen) ratios were essentially the same in the presence and absence of the drug. The same results were obtained with mitochondria from monkey and guinea pig brain cortex, while the liver mitochondria of the same animals showed clear-cut uncoupling in the presence of chlorpromazine. The otherwise-intact reactivity of the brain mitochondria of our preparations was attested to by the fact that 2,4-dinitrophenol uncoupled oxidative phosphorylation in mitochondria from the brain and the liver. Although the ratio of the concentration of mitochondria to that of chlorpromazine in our experiments was clearly arbitrary, it served the purpose of differentiating between mitochondria from the brain and those from the liver. The concentration of the drug relative to that of mitochondria was probably so high as to make it unlikely that the effect found in our experiments with mitochondria from the liver has any bearing upon the *in vivo* action of the drug. One may calculate that the concentration of chlorpromazine used by us per 1.5 mg. mitochondrial N would correspond to 500 mg./kg. in the rat, while Grenell, Mendelson, and McElroy[10] found an increase in ATP concentration in the brain of the rat with 10 mg. of chlorpromazine per kg. In those experiments in which inhibition of succinic acid oxidation by phenothiazine[7] and the uncoupling of oxidative phosphorylation by chlorpromazine in brain mitochondria[12] were observed, the ratio of drug to mitochondrial concentration was probably still more unfavorable than in our experiments.

It was of interest to localize the point of action of chlorpromazine in uncoupling oxidative phosphorylation in mitochondria from the liver. Therefore, the phosphorylation resulting from the oxidation of cytochrome *c* with epinephrine or ascorbic acid as reducing agents was studied. TABLE 3 shows clearly that the phosphorylation resulting from the oxidation of cytochrome *c*

TABLE 3

PHOSPHORYLATION COUPLED TO THE OXIDATION OF CYTOCHROME C

ΔP μmol	O μatom	P:O	2×10^{-4} M Chlorpromazine		
			ΔP μmol	O μatom	P:O
		Ascorbic acid (30 μmol)			
5.3	6.5	0.81	3.1	6.9	0.45
3.4	6.8	0.50	0	7.6	0
4.9	4.9	1.0	1.1	5.1	0.22
		Epinephrine (10 μmol)			
5.8	5.0	1.16	0	4.9	0
4.6	5.6	0.82	0	7.4	0

The reaction vessels contained in addition (in μmol): adenosine triphosphate, 6; phosphate buffer, *p*H 7.4, 30; magnesium sulphate, 30; sodium fluoride, 40; cytochrome *c* (Sigma), 0.3; glucose, 40; hexokinase, 0.4 mg.; liver mitochondrial suspension, 0.5 ml. (average N = 1.7 mg.); and sucrose solution (0.25 M) to a final volume of 3.0 ml. Gas phase, air; incubation at 30° C. with epinephrine for 10 minutes, with ascorbic acid for 20 minutes.

is strongly inhibited by chlorpromazine in mitochondria from the liver. Thes experiments were done with the addition of an excess of cytochrome *c* from horse heart. After having localized part of the uncoupling effect of chlorpromazine on the cytochrome *c* oxidation step, the same experiments wer carried out with mitochondria from the brain, although, on the basis of ou previous experiments, we could not expect to find uncoupling. Despite considerable uptake of oxygen, very little phosphorylation took place, an observation that suggested that added cytochrome *c* was utilized very poorly by mitochondria of the brain.

Although the investigation of chlorpromazine proved to be disappointing as far as its possible mode of action is concerned, the results have significance in indicating the differences between mitochondria from the brain and the liver, not only in regard to the effect of chlorpromazine, but also in regard to the utilization of added cytochrome *c*. It is of interest in this connection that according to a recent suggestion, mitochondria from the brain, in contrast to those from the liver, are able to metabolize glucose by glycolysis.[13,14] It appears possible that these differences between the mitochondria from these 2 organs are structural in nature, and that the effects of chlorpromazine on mitochondria from the liver are primarily an effect on the structure, and only secondarily an effect on oxidative phosphorylation. It may be mentioned here that the sulfoxide of chlorpromazine has been found completely ineffective in uncoupling oxidative phosphorylation in the liver. This oxidation product of chlorpromazine has been found to be excreted after the administration of the drug,[15] and oxidation to the sulfoxide appears to be a general metabolic conversion of phenothiazine, since the sulfoxide has also been detected in the blood of sheep after the administration of unsubstituted phenothiazine.[16]

It cannot be expected that any scheme for the mechanism of the action of chlorpromazine will be evolved from the experimental data reviewed. It is of some interest that the experiments carried out on the whole animal in which the increase of ATP content in different parts of the brain after the administration of chlorpromazine was found are, at least, not in contradiction with the experiments carried out in our laboratory. These demonstrated that chlorpromazine does not uncouple oxidative phosphorylation in mitochondria from the brain at concentrations at which it is effective with mitochondria from the liver. Grenell, Mendelson, and McElroy[10] do not report on the effect of chlorpromazine on ATP in the liver. It would be interesting to know whether an increase of ATP content, such as that found in the brain, was found also in this organ, or if there was a decrease which would be in keeping with our observation on the uncoupling of oxidative phosphorylation in mitochondria from the liver. In both types of experiments there appears to be no interference with the synthesis of ATP in the cortex of the brain. At present, we can only speculate as to whether the utilization of ATP is interfered with by chlorpromazine, whether the drug affects a metabolic pathway not related to ATP, or whether it acts mainly on the structural elements of the cell. The latter mechanism would put chlorpromazine into the group of nonspecific inhibitors interfering with the colloid structure of the cell. It is interesting, in this connection, to note that we have observed that homogenized suspensions of brain, liver, and blood ufficient to absorb chlorpromazine completely out of solution absorb very ittle of the sulfoxide.

The biochemical investigations of chlorpromazine have given 2 results of eneral significance: they have provided evidence for the difference in metabolic ehavior of mitochondria from the brain and the liver, thus suggesting a ifference in structure, and they have shown further that the drug does not nterfere with the formation of ATP by mitochondria of the brain under condiions where it does so with mitochondria of the liver.

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THE CHEMICAL CONSTITUTION AND BIOCHEMICAL EFFECT OF PSYCHOTHERAPEUTIC AND STRUCTURALLY RELATED AGENTS*

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Introduction

Although the number and diversity of pharmacological agents having rathe well-defined effects on metabolism continues to grow, very little effort ha been made to correlate the metabolic disturbance with either the pharmaco logical activity or chemical structure of these agents.

One area in which such investigations have been carried on to excellen advantage is in the case of the cholinergic and adrenergic[1] drugs. Attempts t correlate metabolic disturbances with narcosis have been numerous, althoug the evidence in favor of such a view is not conclusive. In recent years electro microscopy[2, 3] and physicochemical studies[4, 5] have revealed that the mito chondria, which are the cellular sites of energy production, consist of an elabo rate structure surrounded by and intertwined with a lipid (or lipoprotein membrane. The maintenance of the intramitochondrial geometric pattern or of some smaller structure within it,[6] is apparently essential for carrying o oxidative phosphorylation. A wide diversity of agents has been found t inhibit oxidative phosphorylation,[7] but no attempt has been made to classify such agents according to their chemical constitution. In studying the relatio of structure to the inhibition of cytochrome oxidase (a mitochondrial enzyme in a series of organic compounds, the conclusion was reached that the basi structure essential to inhibition was a "diphenyl"† moiety.[8] With the intro duction of chlorpromazine (10-[γ-dimethylaminopropyl] 2-chlorphenothiazine or CPZ) into the field of mental health, interest was renewed in this problem for the structural configurations of this compound suggested that it was relatec to the series of diphenyl compounds studied previously. This relationship wa confirmed and, furthermore, it was found that CPZ was an effective inhibito of oxidative phosphorylation.[9] Certain peculiarities regarding the inhibitory effect of CPZ, such as its complete reversibility, indicated that its reactivity with the phosphorylating complex of mitochondria from the brain resemblec the relationship of the diphenyl compounds to cytochrome oxidase. In th present report, the problem of structure-metabolic activity relationships i re-examined, and a diverse number of diphenyl and related compounds, in cluding reserpine and other tranquilizing drugs, are studied. An attemp also has been made to correlate the metabolic disturbances produced by som of the neurotropic drugs *in vivo*, as well as *in vitro*, to their neuropharmacologica properties.

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† The notation "diphenyl" is intended to refer to compounds with 2 phenyl groups, whether they be tru diphenyls or compounds such as phenyl ether and chlorprophenpyridamine maleate (Chlor-Trimeton).

Methods

The methods used in these studies have been described in detail elsewhere, including the preparation of mitochondria from the brain of the rat, the measurement of oxidative phosphorylation,[10] and the measurement of cytochrome oxidase and adenosine triphosphatase (ATP).[8] For maximal inhibition, it was found advisable to homogenize the drugs thoroughly and then preincubate them with the mitochondrial preparations for 15 minutes at 20° C., especially in the case of hydrophobic substances such as phenothiazine and diphenyl. The *in vivo* studies were performed on adult albino Sprague-Dawley rats (150 to 200 gm.) injected intraperitoneally with 10 mg. of SKF 501. Within 15 minutes the animals showed signs of depression and sluggishness, but no apparent muscular abnormalities. An hour after the administration of the drug, the animals were extremely hypersensitive and hyperactive to stimuli such as a slight jet of air or a snapping of the fingers. At this time the animals were sacrificed by a sharp blow in the lumbar region, and their brains were removed quickly and separated into gray cerebral cortex, cerebellum, and basal ganglia at 0° C. Oxidative phosphorylation was measured on 15 per cent homogenates of the brain segments made up in 0.25 M sucrose containing 10^{-4} M sodium ethylene diaminetetraacetic acid (Versene).

Chlorpromazine (CPZ), Phenergan, SKF 501, and chlorpromazine sulfoxide were generously supplied by Smith, Kline, and French Laboratories, Philadelphia, Pa., and Diparcol was obtained from Roland Fischer of the Psychiatric Institute, Regina, Sask., Canada. The diphenyl curariform agents were kindly supplied by Klaus Unna of the University of Illinois, Chicago, Ill. Phenothiazine, *o*-hydroxydiphenyl, diphenyl, and diphenylamine are products of Eastman Kodak Co., Rochester, N. Y., that were recrystallized from benzene or acetone. Chlorprophenpyridamine maleate (Chlor-Trimeton) was generously supplied by the Schering Corporation, Bloomfield, N. J., and *d*-lysergic acid diethylamide (LSD-25) by Sandoz Pharmaceuticals, Hanover, N. J. Thionol (3-oxy-7-hydroxyphenothiazine) was prepared by oxidizing phenothiazine in 20 volumes of 3 per cent hydrogen peroxide, containing 10^{-6} M copper sulfate as a catalyst, at 80° C. for 30 minutes and then extracting the thionol with amyl alcohol.

Results

Of all the compounds studied, CPZ and SKF 501 were the most effective nhibitors of cytochrome oxidase (TABLE 1). The other CPZ analogues and the diphenyl derivatives inhibited about 30 per cent at 10^{-4} M. Only the phenohiazine derivatives, excluding CPZ sulfoxide, had an appreciable inhibitory ffect on adenosine triphosphatase (ATP-ase) (TABLE 2). 2,4-Dinitrophenol DNP) and, to a slight extent, methylene blue and SKF 501 had an activating ffect on ATP-ase. None of the other compounds activated ATP-ase at maller concentrations.

Oxidative phosphorylation was inhibited by a number of diphenyl and phenothiazine analogues (FIGURE 1). Diparcol and Phenergan produced

TABLE 1

	Conc.	Activity	Per cent inhibitio
	M		
Control	—	2.0	—
Chlorpromazine	5×10^{-5}	1.1	45
10-(2-Diethylaminoethyl)-phenothiazine (Diparcol)	10^{-4}	1.3	35
SKF 1498-A	10^{-4}	1.4	30
Chlorpromazine sulfoxide	10^{-4}	1.8	10
Phenothiazine	10^{-4}	2.0	0
SKF 501	5×10^{-5}	1.1	45
o-Hydroxydiphenyl	10^{-4}	1.3	35
Diphenyl	10^{-4}	1.5	25
2,4-Dinitrophenol	10^{-4}	2.0	0

The effect of "diphenyl derivatives" on cytochrome oxidase of mitochondria from the brian of the rat. Ac tivity is expressed in millimols of reduced cytochrome *c* oxidized/mg. N/10 minutes at 28° C. Each value is th average of 3 determinations agreeing within 5 per cent.

TABLE 2

	Conc.	Activity	Per cent inhibition
Control	—	1.6	—
Chlorpromazine	10^{-3}	1.0	37
10-(2-Diethylaminoethyl)-phenothiazine (Diparcol)	10^{-3}	1.1	32
10-(2-Diethylamino-1-propyl)-phenothiazine (Phenergan)	10^{-3}	1.1	32
Chlorpromazine sulfoxide	10^{-3}	1.5	6
Phenothiazine	10^{-3}	1.3	19
Methylene blue	10^{-4}	1.8	−11
SKF 501	10^{-4}	1.8	−11
o-Hydroxydiphenyl	10^{-3}	1.4	12
Diphenyl	10^{-3}	1.4	12
2,4-Dinitrophenol	3×10^{-5}	2.2	−36

The effect of various compounds on ATP-ase activity of mitochondria from the brain of the rat. Activity expressed in μmol orthophosphate split/10 minutes/amount mitochondria from 10 mg. of tissue. Values are an average of 3 experiments agreeing within 4 per cent.

one half the inhibition of P/O* at twice the concentration of CPZ. The sulfoxide of CPZ was only slightly inhibitory at 10^{-3} M. SKF 501 was twice as effective as CPZ and compares with methylene blue, which is the most potent "uncoupling" agent studied. Phenothiazine, which was virtually water-insoluble, was comparable in effectiveness to CPZ, while thionol, its water-soluble oxidative derivative, was only slightly inhibitory at 10^{-4} M. Among the diphenyl compounds, Chlor-Trimeton and phenyl ether were among the most potent uncoupling agents of the series, while other "diphenyl" analogues though considerably less active, produced some inhibition. LSD-25 caused some inhibition at 5×10^{-5} M. The reversibility of some of the agents was tested and, unlike CPZ, SKF 501 could not be removed by washing the mitochondria with sucrose (FIGURE 1), nor could the effect of DNP and α-naphthol be completely reversed. Reserpine produced over 40 per cent in

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* $P/O = \frac{\mu\text{M P esterified}}{\mu\text{atom } O_2 \text{ used}}$

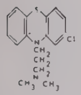
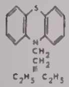
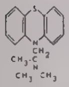
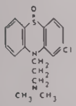
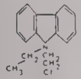
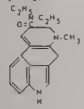
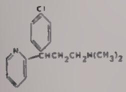
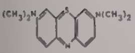
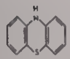
Compound	Concentration	Δ O micro-atoms	Δ P micro-mols	P/O	Per cent of inhibition of P/O
control	--	6.1	16.6	2.7	--
 chlorpromazine	6×10^{-5}	4.3	4.6	1.0	63
 10-(2-diethylamino-ethyl)phenothiazine (Diparcol)	10^{-4}	5.8	11.0	1.9	30
 10-2(diethylamino-1-propyl)phenothiazine (Phenergan)	2×10^{-4}	5.6	11.4	2.0	26
 chlorpromazine sulfoxide	10^{-3}	5.8	14.6	2.5	8
 SKF 501	3×10^{-5}	3.8	4.6	1.2	56
 LSD-25	5×10^{-5}	5.8	12.0	2.2	18
 chlorprophenpyridamine maleate (Chlor-Trimeton)	2×10^{-5}	5.2	9.0	1.7	37
 methylene blue	10^{-5}	4.5	4.6	1.0	63
 phenothiazine	5×10^{-5}	5.8	10.0	1.7	37

FIGURE 1a. The effect of various "diphenyl" compounds on oxidative phosphorylation of rat-brain mitohondria. Each Warburg vessel contained, in the final concentration: 0.008 M K-phosphate, *p*H 7.5, 5×10^{-4} I K-ATP, 0.008 M $MgCl_2$ of 0.015 M KF, 10^{-5} M DPN, 2×10^{-6} M cytochrome *c*, 0.02 M K-pyruvate, 0.001 M -malate, 0.5 mg. yeast hexokinase, 0.004 M glucose, and brain mitochondria from 100 mg. of tissue. The inubation time was 15 minutes, and the temperature was 33° C.

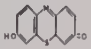
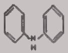
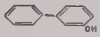
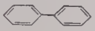
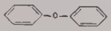
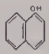
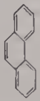
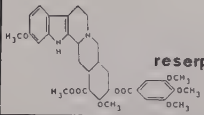
	Compound	Concentration	Δ O microatoms	Δ P micromols	P/O	Per cent of inhibition of P/O
	control	--	6.1	16.6	2.7	--
	thionol	10^{-4}	5.9	14.0	2.4	11
	diphenylamine	10^{-4}	6.2	12.8	2.1	23
	*o*-hydroxydiphenyl	10^{-4}	6.0	10.1	1.7	37
	diphenyl	10^{-4}	6.2	13.1	2.1	22
	phenyl ether	5×10^{-5}	4.0	4.1	1.0	67
	α-naphthol	2×10^{-5}	4.6	7.0	1.5	45
	naphthalene	5×10^{-5}	6.0	12.0	2.0	26
	phenanthrene	5×10^{-5}	5.9	12.0	2.0	26
	reserpine	2×10^{-5}	5.0	8.0	1.6	41

FIGURE 1b. The effect of various "diphenyl" compounds on oxidative phosphorylation of rat-brain mitochondria. Each Warburg vessel contained, in the final concentration: 0.008 M K-phosphate, *p*H 7.5, $5 \times 10^{-}$ M K-ATP, 0.008 M $MgCl_2$ of 0.015 M KF, 10^{-5} M DPN, 2×10^{-6} M cytochrome *c*, 0.02 M K-pyruvate, 0.001 K-malate, 0.5 mg. yeast hexokinase, 0.004 M glucose, and brain mitochondria from 100 mg. of tissue. The incubation time was 15 minutes, and the temperature was 33° C.

hibition in P/O at 2×10^{-5} M, comparing with the most potent uncoupling agents.

In an effort to determine the effect of polar substituents on a single aromatic nucleus, a number of substances resembling the classical uncoupling agent dinitrophenol were tested on oxidative phosphorylation (TABLE 3). Phenol itself was ineffective, while substitution of NH_2, NO_2, and $(CH_3)_2N$ resulted in an increasing order of effectiveness. Dinitrophenol was the most effective inhibitor (45 per cent), but *m*-dinitrobenzene was only slightly less effective (37 per cent). Chloraniline compared with *p*-aminophenol in effectiveness, while only the nitro derivative of benzoic acid was inhibitory.

Since the diphenyl and phenothiazine derivatives were affecting cytochrome oxidase, oxidative phosphorylation was measured in the ferri-ferrocyanide system, which replaces the normal ferri-ferrocytochrome C chain of electron transfer. In such a system, the $\Delta P/2\ \Delta(Fe[CN]_6^{=})$ ratio (corresponding to P/O) was only slightly affected in the case of most of the derivatives studied at a drug concentration producing about a 50 per cent inhibition in P/O (TABLE 4). Methylene blue at a concentration of 10^{-5} M and 2,4-dinitrophenol at 10^{-4} M produced a complete inhibition of the ratio. With 10^{-4} M of SKF 501, a 22 per cent decrease in the ratio occurred.

There are at least 3 important pathways of electron transfer linked to phosphorylation within mitochondria: (1) the oxidation of reduced diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN); (2) substrate phosphorylation involving a phosphoryl-acyl compound, such as phosphoryl succinate; and (3) phosphorylation involved in direct succinate oxidation. In order to determine which of these pathways was sensitive to the diphenyl analogues, the effect of SKF 501 on phosphorylation with different substrates was studied (TABLE 5). Phosphorylation coupled to pyruvate and β-hydroxybutyrate oxidation, which involve DPN-linked phosphorylation exclusively, is inhibited around 50 per cent. A comparable inhibition occurs with succinate as substrate. With α-ketoglutarate, a substrate involving both

TABLE 3

	ΔO μatoms	ΔP μM	P/O	Per cent inhibition
Control	6.1	16.6	2.7	—
Phenol	6.0	16.0	2.7	0
-Aminophenol	6.1	14.5	2.4	11
-Nitrophenol	5.5	10.4	1.9	30
-Dimethylaminophenol	3.5	6.3	1.8	34
m-Dinitrobenzene	5.8	10.0	1.7	37
,4-Dinitrophenol	6.1	9.0	1.5	45
,4,6-Trinitrophenol	6.0	15.4	2.6	4
Aniline	6.1	16.4	2.7	0
-Chloraniline	6.0	14.4	2.4	11
Benzoic acid	6.0	16.5	2.7	0
-Aminobenzoic acid	6.1	16.6	2.7	0
-Nitrobenzoic acid	5.8	14.6	2.5	8

The effect of substitution of various acidic and basic groups in the aromatic nucleus on oxidative phosphorylation of mitochondria from the brain of the rat. Drug concentration was 10^{-4} M.

TABLE 4

	Drug Conc.	$\Delta(\mathrm{Fe[CN]_6^{\equiv}})$	ΔP	$\frac{\Delta P}{2(\Delta \mathrm{Fe[CN]_6^{\equiv}})}$	Per cent inhibition ΔP	Per cent inhibition P
	M	µM	µM			
Control	—	27.5	16.0	1.17	—	—
o-Hydroxydiphenyl	3 × 10^{-4}	17.0	9.3	1.10	42	6
Chlorprophenpyridamine maleate (Chlor-Trimeton)	10^{-4}	28.2	14.7	1.05	8	10
Chlorpromazine	10^{-4}	22.5	12.8	1.13	21	3
Methylene blue	10^{-5}	17.0	0	0	100	100
2,4-dinitrophenol	10^{-4}	9.8	0	0	100	100
SKF 501	10^{-4}	16.6	7.7	0.92	52	22
SKF 501	5 × 10^{-5}	20.0	11.4	1.14	29	3

The effect of diphenyl and phenothiazine analogues on phosphorylation, using ferricyanide as electron acceptor.[10]

DPN-linked and substrate phosphorylation, only a 35 per cent inhibition o P/O is obtained.

In order to determine whether the effect of any of these compounds on phosphorylation could be correlated with their pharmacological activity, particularly in the central nervous system, *in vivo* studies were performed. It hac been reported previously[9] that the region of the basal ganglia of rats treatec with CPZ showed a decreased phosphorylation as compared to the normal while other regions were presumably not affected. Unfortunately, the observation could not be demonstrated consistently, and frequently would be absent completely in severely depressed animals. With SKF 501, however, consistent results could be demonstrated *in vivo* (TABLE 6). In the cerebral cortex a slight but significant increase in oxidation was observed in the treated animal with perhaps a slight decrease in the P/O. No significant change occurred ir either ΔO or P/O in the cerebellum. The region of the basal ganglia (comprised of caudate nucleus + thalamus + hypothalamus) showed a 17 per cent ($p > .001$) in P/O with no change in ΔO. This latter observation could be

TABLE 5

Substrate		ΔO µatoms	ΔP µM	P/O	Per cent inhibition
Pyruvate	C	6.0	16.5	2.7	—
	501	3.8	4.6	1.2	56
α-Ketoglutarate	C	5.2	15.8	3.1	—
	501	3.8	7.6	2.0	35
β-Hydroxybutyrate	C	1.5	3.8	2.5	—
	501	0.8	1.1	1.4	44
Succinate	C	5.8	11.0	1.9	—
	501	4.0	7.9	0.9	53

The effect of various substrates involving different pathways of phosphorylation on oxidative phosphorylation of mitochondria from the brain of the rat. Concentration of SKF 501 was 3 × 10^{-5} M. Values are an average of 2 experiments agreeing within 4 per cent.

TABLE 6

		ΔO μatoms	ΔP μM	P/O	Per cent inhibition P/O
Cerebral cortex	C	6.35 ± 0.50	18.0 ± 0.15	2.84	—
	501	7.00 ± 0.40	19.3 ± 0.20	2.72	4
Cerebellum	C	6.80 ± 0.50	19.6 ± 0.15	2.87	—
	501	6.35 ± 0.60	19.0 ± 0.17	2.98	−4
Basal ganglia	C	5.70 ± 0.50	16.4 ± 1.3	2.88	—
	501	5.55 ± 0.51	13.2 ± 1.0	2.38	17*

The *in vivo* effect of SKF 501 on phosphorylation of various areas of the brain of the rat. 10 mg. of the drug were given intraperitoneally, and the rat was sacrificed 1 hour later. Values are expressed as the mean ± standard deviation, representing 5 experiments.

* Significant to the $p > .001$ level.

demonstrated in every experiment, and in 1 instance as much as a 30 per cent decrease in oxidative phosphorylation occurred.

Discussion

Although an inhibition of oxidative phosphorylation in the central nervous system has been reported *in vivo* with CPZ,[9] the effect has not been consistently demonstrable. With SKF 501, which compares with the most effective uncoupling agents known, it has been possible to demonstrate a significant and reproducible inhibition of oxidative phosphorylation in the region of the basal ganglia. In addition to being a potent inhibitor *in vitro*, the agent cannot be removed completely from mitochondria by washing. Therefore the actual concentration *in vivo* may be expected to be higher than in the case of CPZ. The localization of the effect in this region, which includes the hypothalamus, caudate nucleus, and thalamus, may have a twofold explanation: (1) the absence of a blood-brain barrier in some areas within the region;[11] and (2) a peculiarity in either cellular or mitochondrial permeability. Preliminary observations on mitochondria isolated from the basal ganglia have failed to reveal any particular sensitivity to SKF 501 or CPZ. Permeability factors may differ within the intact organism, however. To what extent the behavioral responses to SKF 501 and CPZ are attributable to the effect on oxidation and phosphorylation is an unsettled problem, but the possibility certainly exists that the drowsiness, depression, and other neurologic disturbances produced by the drugs result from the metabolic inhibition. Especially important n this respect is the fact that CPZ and other psychotherapeutic drugs are beieved to exert their action in the region of the basal ganglia.[12, 13] It may be argued that a 20 per cent decrease in P/O is not adequate to disturb function, but it must be remembered that in the intact structure the concentration of the drug in particular regions of the basal ganglia may have been high enough to produce much higher decreases, and that homogenation of the tissue and urther dilution in the incubating medium resulted in considerable dilution of he drug. Assuming even a random distribution of the drug in the basal ganglia, a 10 per cent homogenate would result in a tenfold dilution. It would eem most logical, therefore, that a disturbance in the main pathway of energy production in such a critical region of the brain as the hypothalamus and thalamus should result in neural disturbances.

Reserpine, which is an effective tranquilizing agent, is an extremely potent uncoupling agent *in vitro*, and preliminary *in vivo* studies indicate that it is also inhibitory to the region of the basal ganglia. The strong inhibitory effect of reserpine is particularly significant in view of the complex chemical structure of the compound. There are structural moieties resembling biphenyl and naphthyl derivatives in addition to harmaline, which is itself an effective uncoupling agent. Preliminary observations[14] have revealed that many indole derivatives, including adrenochrome and derivatives from the urine of phenylketonurics, are formidable inhibitors of oxidation and phosphorylation.

The fact that a diverse number of chemical substances, unrelated structurally to either DNP or CPZ, are inhibitors of oxidative phosphorylation may suggest that many pathways of inhibition are involved. In a previous report[9] evidence was presented to show that DNP does uncouple oxidative phosphorylation in a manner distinct from CPZ, one distinction being the readiness of reversibility with the latter substance as compared with the partial irreversibility of the former. On the basis of the present findings it would appear that, unlike DNP, the phenothiazine and the diphenyl analogues are, at least in part, affecting phosphorylation at the cytochrome-cytochrome oxidase level, insofar as substitution of the ferri-ferrocyanide system for oxygen as the electron acceptor considerably diminishes the inhibitory effect.[8] Since these analogues are inhibitory to cytochrome oxidase, this latter observation is not surprising.

To the degree that the dehydrogenases of flavin enzymes (*fp*) are not inhibited at these levels of drug concentration,[8] the intervention must lie somewhere between the dehydrogenase and the level replaceable with ferricyanide. Precisely where or how these substances act in the following scheme[15] is not at all clear:

$$\text{substrate-DPN-}fp\text{-}b\text{-}c\text{-}a\text{-}a_3\text{-}O_2$$

Unlike antimycin A, which apparently acts between cytochromes *b* and *c*,[16] these substances are inhibitory both to ferricyanide reduction and phosphorylation. As is evidenced by the fact that the inhibition of P/O is appreciably less with α-ketoglutarate as substrate in place of pyruvate or β-hydroxybutyrate, the substrate level of phosphorylation is insensitive to the agent. Apparently the site of inhibition is not specifically related to the oxidation of reduced DPN, since phosphorylation coupled to succinate oxidation is also sensitive. Although in every instance phosphorylation is more sensitive to the agent than oxidation, it is difficult to divorce completely the 2 metabolic processes until more information is available regarding the stepwise mechanism of ATP synthesis and until isolation of the individual enzyme units is achieved.

In the case of the single aromatic series, the degree of inhibitory effectiveness can be correlated to some extent with the dipole moments and other physical properties resulting from acidic and basic substitutions. Compounds such as *m*-dinitrobenzene, *p*-dimethylaminophenol, and 2,4-dinitrophenol are among the most effective inhibitors of the series, and they are also the most hydrophobic and lipophilic. With the exception of *p*-dimethylaminophenol, all the other inhibitory compounds had high dipole moments, usually greater than 4D; 2,4-dinitrophenol had the highest moment and was the greatest inhibitor of the series. The dipole moments of *p*-aminophenol and *p*-chloraniline would be expected to be relatively small and, accordingly, they are poor inhibitors. Resonance would tend to decrease the electronegative effect of chlorine, and hence the vector addition of the complementary (positive) moment of NH_2.[17] The compound *p*-dimethylaminophenol has a low dipole moment, but the presence of the auxochromic $(CH_3)_2$ N considerably complicates the resonance and electromeric characteristics of the aromatic nucleus. To the degree that 2,4-dinitrophenol is moderately acidic ($pK_a = 10^{-4}$) and 2,4,6-trinitrophenol s very acidic ($pK_a = 10^{-1}$), while *m*-dinitrobenzene is not, it would appear that no relationship exists between inhibitory potency and pK_a (or pK_b). A nore systematic study must be done with this series, particularly with regard o restricting resonance and induction by steric hindrance as, for example, by ntroducing substituents in adjacent ortho positions.

The similarity of the phenothiazine and diphenyl series becomes more apparent when their structures are regarded from the standpoint of 3-dimensional nolecular models. Both the over-all dimensions and bond distance between he 2 phenyl groups—or phenyl-pyridyl, as in Chlor-Trimeton or 2-[benzyl-2-dimethylaminoethyl)amino]pyridine (PBZ)—are very similar (FIGURE 2). Perhaps the most outstanding difference in the 2 series of compounds is the oresence in 1 of a thiazine linkage, which would tend to limit rotation of the idjoining phenyl groups and greatly complicate the resonating ring system.

It is not at all clear as to what constitutes the peculiar chemical property of he aromatic derivatives essential to inhibiting the oxidative and phosphorylative metabolism of mitochondria from the brain. Certainly it appears as if he close proximity of 2 phenyl groups is somehow important. Another important requirement is lipid solubility and perhaps water insolubility, insofar as he metabolically inactive sulfoxide of CPZ is highly hydrophilic and lypophobic s compared to CPZ itself. A number of water-soluble, lypophobic compounds, uch as *p,p'*-trimethylaminobiphenyl and *p,p'*-trimethylaminobiphenyl ethane, both potent curariform agents, were found to be ineffective as uncoupling agents. Polar substitutions on the benzene rings, such as OH and Cl, lo not appear to be essential, although they increase the activity within a given series. Compare, for example, CPZ, Diparcol, and Phenergan. The oresence of a thiazine linkage between the phenyl groups is apparently not ssential to either phosphorylation or to cytochrome oxidase inhibition. Substitution of the positive auxochromic $(CH_3)_2$ N on both phenyl groups of ohenothiazine to form methylene blue greatly enhances the inhibitory effect n P/O. The presence of this substituent enhances and increases the resonance tructures while converting phenothiazine into a dye having a high affinity for oroteins and an oxidation-reduction potential comparable to that of the flavin nzymes. It would appear, however, that insofar as the phenothiazine and aphthyl derivatives would be more likely to assume a coplanar configuration, oplanarity is perhaps essential to inhibition.

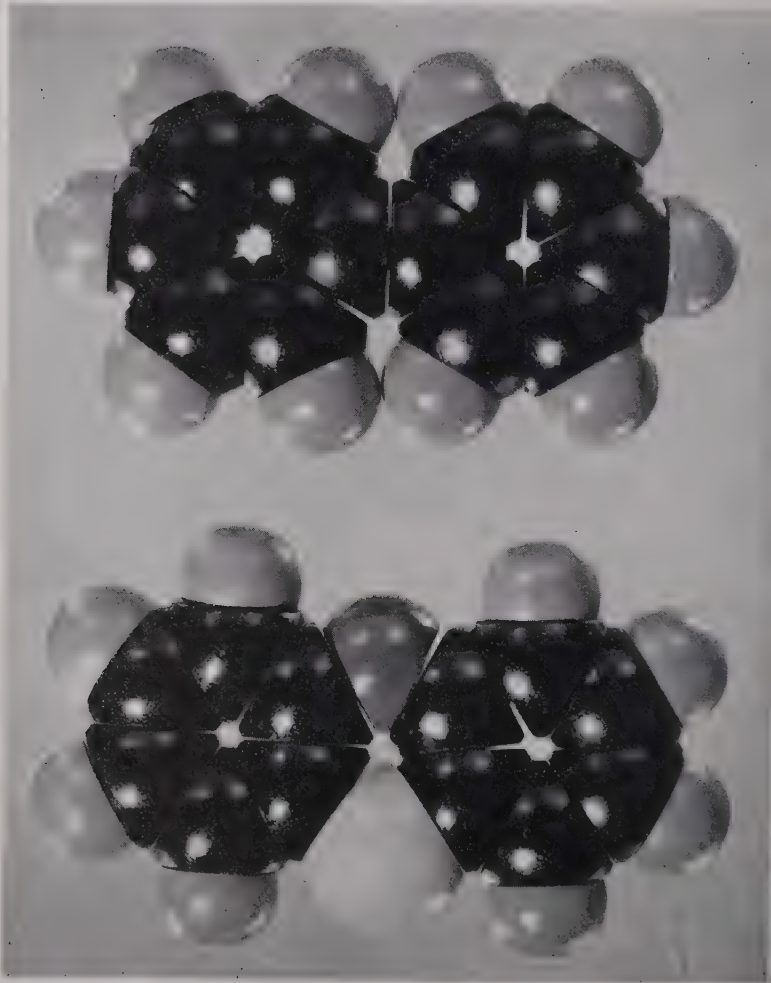


FIGURE 2. The similarity of the phenothiazine series (bottom), to the diphenyl series (top), as illustrated by 3-dimensional molecular models.

A great number of the compounds in the present study, other than the psychotherapeutic and antihistaminic drugs, are of pharmacological interest. Phenothiazine, DDT, naphthalene, and the nitrophenols are effective anthelminthics. In studies with an extensive series of analogues of the insecticide DDT, toxicity was correlated with a high lipid-water partition coefficient or, rather, lipid affinity. Halogen substitutions in the order of increased electronegativity (F < Cl < Br) on the benzene ring increased, while polar substitutions decreased toxicity.[18] DDT is an effective inhibitor of both oxidative phosphorylation and of cytochrome oxidase, such an effect being predictable on the basis of its chemical structure and physical characteristics. No attempt has been made to compare the insecticidal action with the degree of enzymatic inhibition, but this would seem to be a worthwhile project. It is particularly noteworthy that phenyl ether, among the most potent uncoupling agents of mitochondria from the brain, constitutes the basic structure of thyroxine, which has been reported[19] to be an uncoupling agent, although less effective than phenyl ether. To what extent the pharmacological effect of any of these agents can be correlated with their effect on oxidative phosphorylation is not certain, but certainly some degree of correlation seems highly probable.

The most probable type of reaction between this class of compounds and the enzyme systems appears to be through van der Waal forces that may be sufficient to disturb the delicate balance of enzyme patterns within mitochondria. Since many of the compounds studied do not possess reactive chemical groups, and their inhibitory effect is so readily reversible, it would appear that chemical adsorption is not of primary importance. Although aromatic compounds would be expected to have high van der Waal forces and may, for example, increase the affinity of substances for proteins,[20] it is not at all clear what the peculiar role of the aromatic nucleus could be. Both the shape and size of the diphenyl and phenothiazine molecules are similar, and it is conceivable that the parameters are such as to bring about occlusion of the free lypophilic spaces within the mitochondrial complex, a theory recently proposed by Mullins[21] for narcotic action. Such occlusion can occur either at the outer mitochondrial membrane and thus influence extramitochondrial transport, or intramitochondrially, in such a way as to affect the transport of electrons or molecules either by chemical means or physical distortion of the structural relationships of enzymes and cofactors. Lehninger[6] has succeeded in isolating an intramitochondrial complex from mitochondria from the liver that is capable of carrying on oxidative phosphorylation. Presumably the mitochondrial membrane is absent from such a system as well as from the bulk of enzymes not directly related to phosphorylation. Such an agent as thyroxine, which is inhibitory to the oxidative phosphorylation of intact mitochondria, is inactive in such a system, while 2,4-dinitrophenol is equally effective on both systems. This difference would seem to indicate that thyroxine uncouples by virtue of its action on the mitochondrial membrane. Since we have not yet succeeded in isolating such a system from mitochondria from the brain of the rat, it remains to be determined which of the agents studied acts at the mitochondrial surface. Until more is known about such structural interrelationships and the detailed mechanisms of phosphorylation, the nature of the inhibition is a matter of sheer speculation.

Recently, Fischer[22] has demonstrated a correlation between the affinity of various drugs to keratin and the ability of these compounds to produce psychoticlike symptoms. Astbury[23] has proposed that adjacent amino acids in proteins such as α-keratin may be folded into 6-numbered rings, the closure being affected by a lactam-lactim transformation and a keto-enol change Both the molecular shape and the dimensions of such hexagonal configuration of polypeptides are such as to suggest the possibility that aromatic structures by such means as the van der Waal forces and hydrogen bonding, may becom lodged in the interstices and interfere with metabolic transformations. Insofa as such physical and chemical properties as solubility, acidity, and nucleophili substitution are determined by the resonance and electrophilic properties o aromatic compounds, it is not inconceivable that electron transfer or oxidation reduction potentials within the mitochondrial chain of enzymes could b interrupted or disturbed by the same mechanism. The inhibition of oxidativ phosphorylation by the electrical excitation of mitochondria from the brain ma be caused by a similar mechanism.[10] A knowledge of the interactions of suc compounds with proteins must await a fuller understanding of the structure an chemistry of proteins.

Future work in this laboratory is contemplated along the lines of studyin the affinity of aromatic compounds for specific proteins and enzyme system with an effort toward correlation of the electrophilic and nucleophilic propertie of the compounds with enzymatic inhibition. Perhaps, through such studies a rational basis can be established for the development of neurotropic drugs particularly those of importance in the field of mental health.

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MECHANISMS OF ACTION OF PSYCHOTHERAPEUTIC AND RELATED DRUGS*

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A number of suggestions as to the mechanism of action of many of the new "psychiatric" drugs have appeared in the literature. Some of these are based on fond attachment to long-existing theory, and others deal with more recent observations. In any case, none of these suggestions thus far has been able to answer all the necessary questions. Some of the so-called classical explanations are based on interference with blockage or stimulation of "adrenergic" or "cholinergic" systems[1]—with essential deviation from normal equilibrium levels of "transmitters." Evidence would suggest that these factors are involved, but that they may not be the primary focus of action of various substances. If they are involved more importantly in the basic steps, it becomes necessary to deal with recent evidence that forces us to the conclusion that there are a large number of different transmitter substances in the central nervous system, and that, consequently, an unusually large number of specific reactions to the drugs employed must exist. Somehow this possibility does not seem too impressive. As we shall see, there is reason to believe that one can construct a theory of action that does not rely on the existence of so many different and specific reactions. Although a wide spectrum of "trigger" mechanisms may play an important role in certain network responses, it may be that different substances do not necessarily become directly involved with these "triggers" but may be more closely dependent on certain structural differences, relative, in part at least, to receptors and the special properties of receptor sites. In other words, there is reason to believe that whatever neurohumors may be involved, in whatever way, certain structural membrane factors must be considered, that is, of membranes at synapses, and around neurons as well as within them.

Recently a number of investigators have suggested that a wide variety of drug actions can be interpreted as consequences of differing effects on the ascending reticular activating system. It would appear, certainly, that this system does play a role in these mechanisms, but that this is not the over-all answer that we seek. One of the greatest points of interest of this system, however, is that it serves a major role in considerations relating certain types of drug action to the interaction of several areas of the brain at different levels. It is not enough to observe the involvement of "arousal"—it is essential to be certain at what level or levels the "arousal" system has been affected. In fact, it is essential to be certain of the area or areas of the brain that are the primary sites of action.

In the light of such considerations, a series of studies was undertaken in our

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TABLE 1

ATP[*] WITH REPEATED INJECTIONS OF CHLORPROMAZINE[†]

Area	Control	1 Dose	8 Doses, 8 days	10 Doses, 10 days	15 Doses, 15 days
Cortex	75	105	136	84	82
Thalamus and hypothalamus	63	94	173	135	105
Cerebellum	72	98	97	94	94
Medulla	68	86	84	86	77

[*] Mg./gm. wet weight.
[†] Ten mg./kg.

aboratories, directed toward some clarification of the effects of depressants and stimulants on neuronal groups in particular cerebral areas. Two points were regarded as of major importance: (1) the possible demonstration of chemical correlates of activity and changes in activity of *selected* cell groups; and (2) the further demonstration of the primary action of specific agents on specific cerebral areas as reflected by the response of such areas to stimulation.

The first group of experiments dealt with the effects of chlorpromazine on adenosinetriphosphate (ATP) levels in selected areas of the brain.[3] The decision to measure ATP was based on several factors, among which was the fact that previous studies on the mechanisms of narcosis had suggested that analysis of ATP utilization would be of value.[4] It was found that chlorpromazine injected into rats (I. P.) in doses of 10 and 50 mg./kg. produced a slight ncrease in ATP in the medulla and cerebral cortex, a somewhat larger one in the cerebellum, and a most dramatic increase of 130 per cent (with one dose of 50 mg./kg.) in samples designated as a mixture of thalamus and hypothalamus. In more recent analyses, values have been obtained for both thalamus and hypothalamus. After one dose of 50 mg./kg. the ATP in the hypothalamus ncreased 158 per cent (average of a series of samples) and in the thalamus 145 per cent. As compared with these values, the increase in cerebral cortex was was only 36 per cent and, in the medulla, 27 per cent.

For purposes of comparison, similar measurements were carried out following njections of dechlorinated chlorpromazine (Sparine[*]). With a dose of 50 mg./kg., an increase in ATP was apparent, but was of much smaller magnitude han that observed with chlorpromazine. A dose of 10 mg./kg. was not ollowed by any significant thalamic or hypothalamic shift. When the dose was raised to 75 mg./kg., the hypothalamic ATP increased approximately 100 per cent, but there was still no significant increase in the thalamus. In this nstance, however, the cerebellum showed an increase in ATP that far surpassed hat obtained with chlorpromazine. There appear to be some behavioral differnces, as well, between the animals given these two drugs, but further studies must be carried out before very much can be reported in this regard. No hronic studies have been made, as yet, with Sparine, but repeated injections f chlorpromazine appear to be most interesting (TABLES 1 and 2). On the 0 mg./kg. dose (1 injection per day) the animals were kept going only four ays, because their physical state by that time was so extremely poor several

[*] The Sparine was kindly supplied by Wyeth Laboratories, Inc., Philadelphia, Pa.

TABLE 2

ATP* WITH REPEATED INJECTIONS OF CHLORPROMAZINE†

	1 Dose, 1 day	4 Doses, 4 days
Cortex	150	140
Thalamus and hypothalamus	144	176
Cerebellum	154	167
Medulla	107	99

* Mg./gm. wet weight.
† Fifty mg./kg.

died before the fourth day. In the light of the ultimate reversal of the ATP increase seen with the repeated 10 mg./kg. dose, however, it is of interest to note that on the 50 mg./kg. dose, by the fourth day, reversal was just beginning to be apparent in the cortex and medulla. The thalamic-hypothalamic region and cerebellum still showed some increase. What is more important is that the subcortical centers show a higher, more slowly reversing ATP level than any other areas measured. It is believed that these shifts with chronic dosage are related to an adaptation or tolerance phenomenon.

It may be worthy of mention, at this point, that a study of levels of nucleic acids in various parts of the brain is being undertaken. As an adjunct to this study some preliminary measurements have been made of nucleic acid levels in several areas after injection of chlorpromazine in both dosage levels used in the earlier ATP experiments. Unfortunately, no measurements are available as yet on the hypothalamus, but initial results indicate that in the treated animals (after 1 dose) there is a significant drop in nucleic acids in the cerebral cortex and the cerebellum.

In any case, a definite chemical change in specific cell groups is apparent. This change is associated with a marked change in behavior. Again, we are confronted with a series of difficult questions:

(1) Is the ATP change related to shift in the level of cellular activity?

(2) What, if any, are the implications of a decrease in subcortical activity as related to the over-all effects of the drug?

(3) What factors relate directly to the cellular selectivity or specificity involved?

A group of experiments has already been carried out and partially reported[5-8] dealing with the correlation of ATP level with neuronal activity. There is no question (for example, as shown in TABLE 3) of the inverse relationship that exists; namely, the greater the activity, the lower the ATP level or vice versa. What mechanisms are operative within this framework still remains to be determined, but the correlation exists, and certain evidence[4] even allows us to begin to understand the causal relationship or sequence involved. Other investigators using different techniques have presented some results which agree quite well with these.[9-12]

These effects of chlorpromazine and Sparine would seem to indicate that they induce a state of decreased activity in selected subcortical areas. It is scarcely necessary to dwell upon the disturbance of cortical-subcortical equilib-

TABLE 3

ATP OF THE SUPRASYLVIAN GYRUS OF THE CAT

Experimental group	ATP (in μg./gm. wet wt.)	Percentage of change from unanesthetized control
Unanesthetized (control)	214.45	
Electric shock* tonic phase	249.66	+16%
Anesthesia (Nembutal)†	383.70	+78%
		Percentage of change from anesthetized control
Anesthesia + Metrazol convulsions‡	153.80	−60%
Anesthesia + electric shock	455.50	+18%

* Ninety V.; 0.2 seconds; 60 cycles.
† Dosage = 40 mg./kg. (I.P.)
‡ Dosage = 60 mg./kg. (I.M.).

rium systems that such altered activity levels would create. Certainly marked changes in behavior have been shown to occur in both animals and man when these feedback loops have been disturbed in any way. In fact, it is not even too improbable to suggest that such imbalances play a major role in the over-all picture of a psychosis such as the manic-depressive state. For the moment, however, it is necessary to look further at this "regional" concept, especially in the direction of any other evidence that would suggest that drugs that change behavior may not or do not all act on the same areas of the brain.

With this suggestion in mind a series of experiments was begun in an effort to obtain, if possible, some information concerning regional effects of insulin and lysergic acid diethylamide (LSD-25).* In the case of both drugs the effects of both systemic intravenous injection and local cortical perfusion (by microcannulation, directly into a small pial vessel) were examined in cats. The effects were "assayed," according to observed changes in the characteristics of the evoked cortical response to direct electrical stimulation. The technique of local cortical perfusion serves two purposes:

(1) It allows for exposure to many substances of small local areas of the erebral cortex that can thus be kept under the chemical control of the investigator as long as desired. It is possible, of course, to let the blood flow back nto the area at will, reducing the perfusion pressure.

(2) By perfusion of the control solution (which contains the necessary actors to maintain the normal rate of oxygen consumption and electrical esponse of the cells) the area can be kept in neural contact with the rest of he nervous system while isolated from any direct circulatory effects.

FIGURES 1 to 5 show the first results of these experiments. In all records, egativity at the recording electrode is signaled by an upward deflection. All esponses were obtained from the suprasylvian gyrus of cats anesthetized with pentobarbital sodium. The results are summarized as follows:

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* The LSD-25 was very kindly supplied by Sandoz Pharmaceuticals, Hanover, N. J.

(1) Systemically injected insulin in the doses used markedly facilitates th cortical evoked response (this is also true of the transcallosal response).

(2) This facilitation is not produced by insulin perfused directly into a loc cortical area.

(3) In the decerebrate cat, in which the ascending reticular activating syste has been cut in the midbrain, the insulin facilitation of the cortical response i *not* seen.

(4) LSD-25, in the doses employed, inhibited the cortical response whe injected systemically and when locally perfused into the cortex.

(5) The inhibition induced by LSD-25 was observed even in the case o interruption of the ascending reticular activating system.

These findings indicate that insulin does not act directly on the cerebra

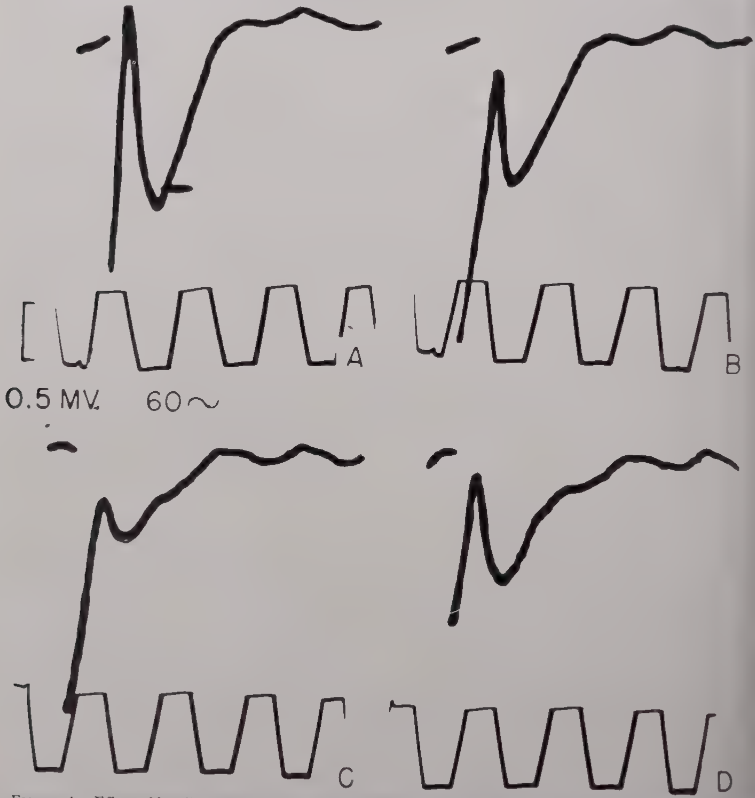

FIGURE 1. Effect of locally perfused (25 μg./cc.) LSD-25 on the evoked cortical responses in a suprasylvian gyrus, anesthetized cat. FIGURE 1A shows the control response; 1B and C, the responses after shifting to LSD perfusion (spike response is barely visible in 1B and completely gone in 1C); and 1D, the recovery after shiftin back to the control perfusate.

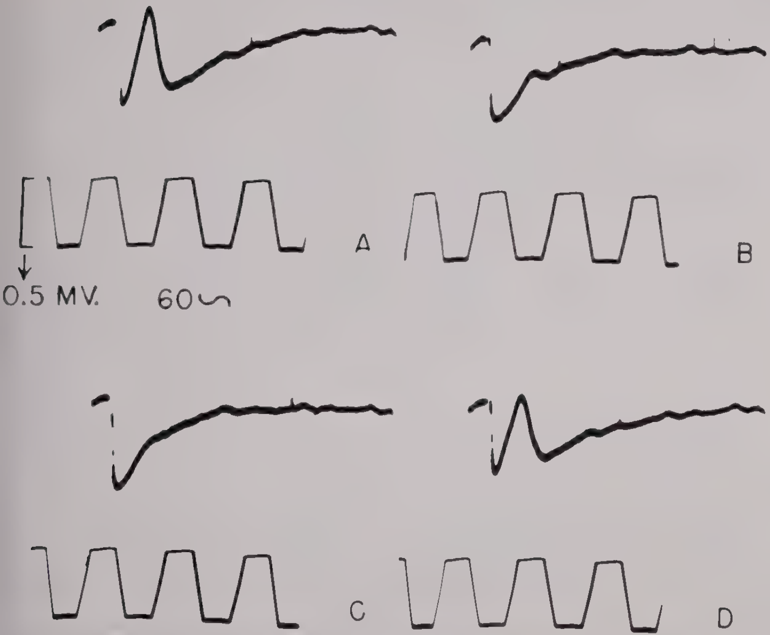

FIGURE 2. Effects of locally perfused LSD-25 on the evoked cortical responses in a suprasylvian-gyrus, nesthetized cat. FIGURE 2A shows the control response; 2B, the inhibition during perfusion with 2.5 μg./cc.; C, the marked inhibition during perfusion with 25 μg./cc.; and 2D, the recovery with the control perfusate.

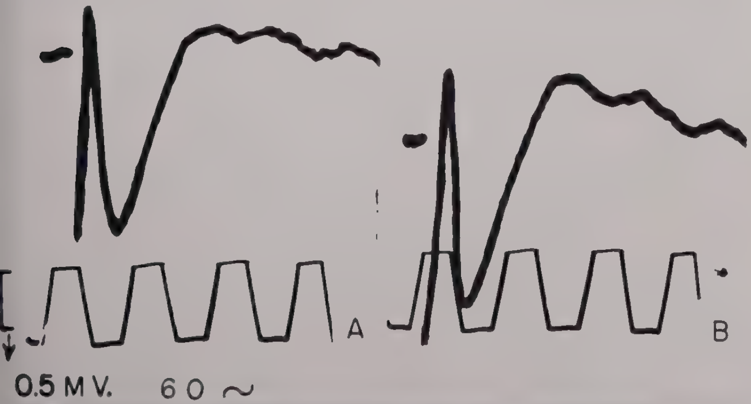

FIGURE 3. Effect of locally perfused insulin (2 units/cc.) on the evoked cortical responses in a suprasylvian-yrus, anesthetized cat. FIGURE 3A shows the control response and figure 3B the response during the perfusion f insulin.

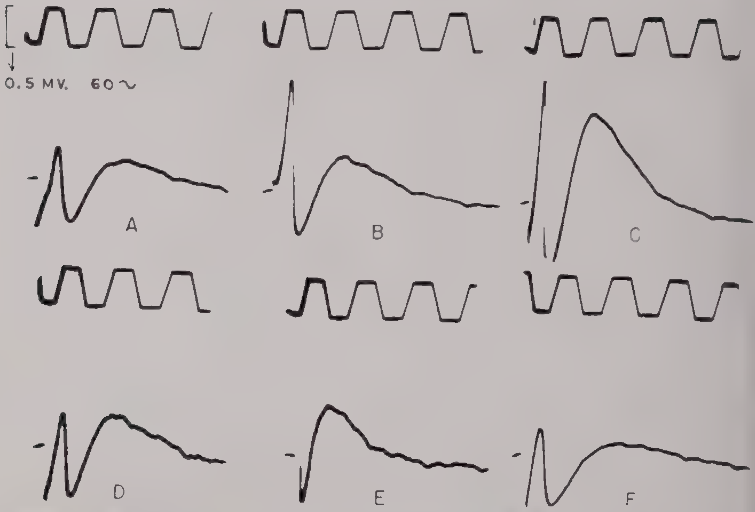

FIGURE 4. Effects of insulin (500 units/kg. I.V.) on the evoked cortical response of the normal and the undercut cortex. FIGURE 4A shows the control response in the right suprasylvian gyrus; 4B, the response in the right suprasylvian gyrus after the injection of insulin; 4C, the response in the suprasylvian gyrus still later after insulin; 4D, the control response in the left suprasylvian gyrus; 4E, the response after undercutting the left suprasylvian gyrus, before the response has completely recovered and before the intravenous (by femoral vein) injection of insulin; and 4F, the response obtained in the recovered left suprasylvian gyrus after the injection of insulin.

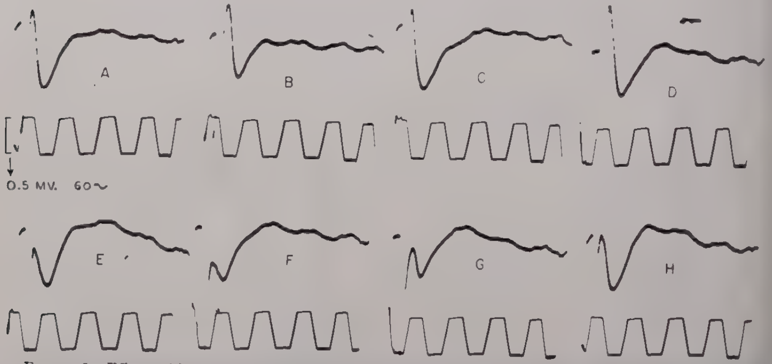

FIGURE 5. Effects of insulin (1600 units, I.V.) and LSD-25 (10 γ/kg. I.V.) in a cat decerebrated at the midbrain. FIGURE 5A shows the control response; 5B, C, and D, the responses after the decerebration and the injection of insulin; 5E and F, the developing inhibition consequent to the injection of LSD; and 5G and H, the recovery.

cortex. On the other hand, LSD-25 can do so. The fact that no facilitation has been seen, as yet, with lysergic acid may be due to the anesthesia, as pointed out by Purpura.[13] It is of interest to note that under the conditions of these experiments, the most striking effect of LSD-25 was on the "dendritic" spike. This suggests agreement with Purpura's report of lysergic acid's axodendritic selectivity.[14] The present observations more or less force the working assumption that whatever the effect of insulin on the brain, it is accomplished by action on subcortical centers via the ascending reticular activating system. LSD-25, however, presumably can act more directly on the cortical areas that one supposes would be involved in the production of hallucinations. This does not mean to imply that this is the sole mode of action of LSD-25. As further information is obtained, it will become necessary to fit these observations into a reasonable hypothesis that also takes account of the other reactions hat must be going on simultaneously. Among such possibilities might be included the suggestion of B. B. Brodie that serotonin, of which he finds massive amounts in the hypothalamus, could act as a transmitter. It is not impossible hat insulin could alter the normal picture of serotonin activity in the hypothalamus in such a way, for example, as to interfere with transmission of inhibitory impulses to the cortex.

Future experiments will have to deal more directly with such aspects of the problem.

One of the major questions these considerations bring to mind still remains; namely, that of the actual cellular selectivity involved. Why do certain drugs act on cortical cells, others on thalamus, still others on hypothalamus, etc.? In the light of our previous work on narcosis[15] and the recent papers of Mullins on physical mechanisms in narcosis[16] and olfaction,[17] it is suggested hat the crucial problem here is concerned with the structures of neuronal membranes in different areas of the brain and their relationship to the physical properties of molecules to which they are exposed. The membrane is no onger merely a research concept. The pictures of Wyckoff and Young[18] present direct evidence of a structure whose existence was previously determined largely by polarization, capacitance, and permeability measurements. Whatever the nature of this membrane may be, its role is primary in all the processes that take place at the cell surface. In fact, it would appear that the major task of the nonrespiratory intracellular mechanisms is that of maintainng and resynthesizing the membrane during the course of the functional cycle f the neuron. It should be pointed out that the theoretical considerations here involve not only the "membrane" as a concept, but also the concepts of nzyme and receptor. Mullins has suggested that the membrane may be an nzyme and may be a receptor, the former dealing primarily with rates of reacion and the latter with rates of ion transfer. Obviously, there may be several ypes of enzymes and receptors involved in any particular activity. If the neuronal surface structure or membrane is considered to have a macromolecular tructure with interspaces of a particular size, as well as a certain number of pecific types of receptors,[16, 17] then certain suggestions that could explain he action of specific molecules on selected cell groups can be made. The details of this type of approach to the action of psychotherapeutic and related rugs will appear elsewhere,[19] but a few points are of immediate interest.

Very suggestive evidence has been presented from several points of view:

(1) ". . . the excitatory action of molecules is by virtue of their ability to

produce a local disorder in the oriented molecular structure of the cell men brane."[17]

(2) There are many instances in the literature supporting the concept the occurrence of behavioral changes directly associated with reorientation particular molecules. It has been shown, for example, that one isomer hexachlorocyclohexane acts as a narcotic, while another isomer is a convulsan Slight reorientations of the reserpine molecule render it ineffective either as tranquilizer or an antagonist of serotonin. Further, azacyclonol (Frenquel), well-known blocking agent, is the gamma isomer of alpha-(2 piperidyl)benzohydrol hydrochloride (Meratran), a stimulator. Nor are the steric propertie of the steroids to be overlooked, especially if one considers the fact that wit a slight reversal (that is, turning it upside down) the lysergic acid molecul closely resembles those of certain steroids, some of which also possess hallucinogenic properties.

In general the problems of local selectivity appear to be basic to the effect produced as a result of deviation from the normal regional interaction of severa levels of the central nervous system. Certainly a great deal more must b learned of the mechanisms of action of specific drugs on specific cells, but ther appears to be no doubt that at least some major facets of the problems o normal and abnormal behavior are clarified by this type of investigation.

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THE IMPLICATIONS OF PSYCHOPHARMACOLOGY IN THE ETIOLOGY AND TREATMENT OF MENTAL ILLNESS

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I am sure it is neither original nor necessary to observe that the great interest that was aroused by the conference on which this monograph is base stemmed not only from the fact that the papers here presented may contribut to a fundamental understanding of the mechanisms of the nervous system, bu also from the fact that the work reported appears to have important implications for the great problem of mental illness. In the atmosphere of interes and optimism created by advances in psychopharmacology and in the hope an expectation that this field may contribute to an ultimate solution of th problem it may be of value to review briefly part of the history of the etiological theories concerning mental illness. For, just as the individual learns from his own past experience, so does science profit by reviewing the history of scientific theory, including its errors and premature convictions as well as it precocious strides.

The trends in psychiatric thought have shown wide swings from one extreme to another, wider perhaps than those that characterize many other areas of scientific inquiry. It is the inherently mysterious nature of the interactions between the spheres of mind and body and the great tragedy and ubiquity of mental illness that have provoked the urge for the solution of the problem represented by this illness, and the urge for a solution of it has increased, sometimes disproportionately, the gap between theory and fact.

The first era of psychiatric theory, embracing the earliest of historical writings, may be called the Period of Demonology. The Ebers Papyrus, written about 1550 B.C., the chief source of information concerning Egyptian medicine, describes mental disorders as dependent upon evil spirits. An early Hindu work, the Ayurveda, of the 14th century B.C., classified mental illness into 7 varieties, according to their supposed etiology. Five of these were the result of angers of specific devils, one was attributed to anger of the gods, and one to anger of the spirits of dead men.

In the rational era of Greek civilization, mental illness came to be regarded by most of the Greek physicians and philosophers as stemming from natural causes. Pythagoras, in 500 B.C., was probably the first to place the center of intellect in the brain and to consider mental illness a disorder of that organ. The great Hippocrates elaborated a number of incredibly relevant theories regarding the causes and nature of mental illness. It is interesting to speculate on how frequently Hippocrates might point out, if he were to read these pages today, that many comments in them had been anticipated by his own prior and pertinent observations. Perhaps he will permit me to speak in his behalf. Hippocrates considered the brain to be a gland secreting substances for distribution to the rest of the body. Retention of these substances in the brain by one or another pathological process could smother that organ and produce certain forms of mental aberration. In another context, he wrote that air was important for intelligence, that various organs could participate in the intellect in proportion to their consumption of air, and that the brain was the highest in this respect. Decreases in the availability or utilization of air produced mental changes and could produce certain of the mental illnesses. Hippocrates recognized the composition of the blood as an important factor in preserving the integrity of the mind, and he stated that as long as the blood maintained its normal composition, intelligence would remain intact. His best known theory regarding one rather well-defined mental illness was that for hysteria, which he regarded as the result of suffocation of the uterus. One wonders why, after the effects of lysergic acid diethylamide and of serotonin upon the uterus of the guinea pig were observed, no appropriate theory for the mental effects emerged.

In the Middle Ages in Europe there was a reawakening of the demoniacal theories for mental illness, except that now the vague category of evil spirits was replaced by a more specific and better described mechanism—Satan. Probably in reaction to the Dark Ages and their inability to solve the enigma of mental illness, the pendulum once more swung back to a more material or naturalistic approach. The Age of Reason and Empiricism, which began with Francis Bacon in 1600, led quite directly to the materialism of the 18th and 19th centuries, which had expected results upon psychiatry. No aspect of human behavior or human emotion was safe from explanation by the often quite naïve materialistic philosophies of that era. Consciousness, in the words of one writer, was the secretion of the brain, as bile is the secretion of the liver. Mental illness of all kinds, even criminality and, for that matter, juvenile delinquency, I suppose, were attributed to a gross organic alteration of the brain, determined, in great measure, genetically. The theories of etiology were in tune with the times. This was the period when the exciting sciences were the anatomical and the bacteriological, and these sciences determined the heories. Mental disease was believed to be the consequence of a demonstrable pathologic lesion in the brain or the result of invasion by one or another of the newly discovered microorganisms. There was great optimism, and many authorities confidently predicted the emptying of all hospitals for the mentally ll.

This did not happen, but theories continued to pile upon theories, most of hem naïve and premature and never shown to be valid—autointoxication, erebral ischemia, defective oxidation in the brain, and various disorders of the ndocrine system. Some of these speculations were based upon sound and verifiable evidence. Some of the mental illnesses are characterized by readily dentifiable somatic alterations, but these are all too frequently found to be the esults of the mental illness rather than its cause. In any case, the various organic etiologies presented at the time remained only theories supported by a ew observations, dependent upon somewhat hasty reasoning, and rarely capable of confirmation. These theories seldom led to beneficial therapy.

Then came Sigmund Freud and the psychoanalytic school that followed him. This school emphasized the tremendous influence of early experience and of interpersonal relationships upon behavior and personality. Although Freu never lost sight of biological factors underlying the psychological, some of h followers, in reaction perhaps to the disappointment of the premature organ theories, tended to lose sight of and to deny the role of biological mechanisms i mental illness.

Today we appear to be on the threshold of another swing of the pendulun a swing that seems to be impelled by a number of factors. We have seen witl in our own lifetimes two important mental illnesses solved in terms of biolog cal techniques: paresis on an infectious basis, and the mental manifestations pellagra on a nutritive and, later, on a biochemical basis. There has also bee considerable disillusionment and some reaction to the more extreme of th psychodynamic concepts that chose to neglect the possible participation somatic mechanisms in diseases of the mind. There has been the confirmatio of old, and the reporting of new, data that emphasize, in a manner that appea to me to be quite inescapable, the importance of genetic factors in certain the mental illnesses. Finally, there has been a great reawakening of interes in the old science with the newly coined name of "psychopharmacology."

If we have learned anything from the history of psychiatric theory, it is tha these theories have erred seriously by becoming too dogmatic, too comprehen sive, and, most important of all, too simple. It is quite improbable that an of the mental illnesses are entirely explicable in biological or sociological psychological terms, for it is quite apparent that these diseases represent a interaction among these spheres.

Pharmacology has always recognized that the effects of a drug are neve produced entirely by the drug itself, but are dependent upon an interactio between the drug and the organ or system affected. This is true even of th heart, which has relatively few degrees of freedom—it can do little more tha beat or stop beating, speed up or slow down, become irregular or regula Even in that organ, digitalis produces different effects upon different heart with different types of function. If we turn to the brain, or mind, with it almost infinite degrees of freedom or variability of response, we are forced to realization of the importance of the structure or the substrate upon which drug acts, for the brain is a substrate that is infinitely more complicated tha those dealt with in other fields of pharmacology. In this case we deal wit personality, or mood, or affect, which to me, at least, are merely convenien terms for that highly complex and most individualized physicochemical syste which reflects the totality of constitution plus the storehouse of past experienc mingling in the arena of present awareness. At one time or another we hav all participated in an experiment in which a standard dose of a drug, alcoho is given to a large number of individuals. The responses are much mor varied than are those to digitalis or quinidine. In fact, there are as man different responses as there are individual subjects and, more than that, eac subject has a wide range of possible responses depending upon his particula mood or the circumstances under which the drug was administered. Just a the pharmacology of the cardiac drugs must include physiology and biochemis try in its perspective, so psychopharmacology must include not only thes isciplines, but also the sciences that deal with personality and behavior (at east until the biochemist appears who will explain these in chemical terms).

The problems of psychopharmacology are many, and they are difficult. It not easy to find suitable models and meaningful screening techniques. The eart of the cat may be a satisfactory assay for digitalis, but who would insist at the cat's motivation and personality, or even its behavior, are suitable odels for those of man? The behavioral pharmacologists contributing to is monograph are well aware of that problem, and they have approached it, believe, with appropriate discretion.

One concept emphasized by some of the contributors to these pages can ardly be overemphasized. The mere fact, no matter how often substantiated, at a drug has a particular effect upon a certain system under specific circum-ances does not constitute evidence that this is the mechanism by which it roduces its clinical effects. A hypothesis that rests only upon observations at do no more than fail to contradict it is poorly supported.

Some of the biochemical mechanisms by which these drugs may act have een reviewed. James A. Bain has exhaustively and dispassionately reviewed vidence supporting the belief, based upon brain homogenates, or brain slices, r even stimulated slices, that the site of action of LSD is in the oxidative etabolism of the brain. The report made by Louis Sokoloff, however, that the brains of actively hallucinating subjects no over-all change in cerebral xygen consumption is detectable must have some relevance. Although one an propose localized changes in oxidation as a possible reply, it appears to me at a system so universal and fundamental as that of oxidation is not too likely be the primary site of action of a drug that exhibits mental effects of such reat specificity with little tendency to become more general as the dose is creased. It is possible that the primary effect is in oxidative phosphorylation, hich has now succeeded oxidation as the exciting and stimulating point the conversion of biochemical matter to functional energy. It is worth ecalling, however, that phosphorylation is still a universal process, occurring probably every cell in the body, and that it is still difficult to see how a drug hose sole effect involves this ubiquitous process can have such highly spe-alized effects upon the organism.

The fascinating theories relating lysergic acid diethylamide, mental illness, d its pharmacotherapy to brain serotonin received something of a jolt from e data of J. H. Gaddum and Ernst Rothlin. I am sure that these data will rovide many opportunities for the revision of some of these theories.

Many neurophysiological data have been presented on the action of hallu-nogens in the central nervous system. There is little doubt about the resence of these effects, but Edward V. Evarts raised a question as to whether ese were the important effects, and Julia T. Apter, with evidence that hallu-nations, like beauty, may be in the eye of the observer, amply corroborated s reservations.

I am quite sure that no theory on the mechanism of action of either group of e drugs described throughout this monograph will receive general approval. for one, however, am by no means discouraged. When we realize that neither

the biochemical nor the physiological mechanism of action of alcohol or ba
biturates has been established after years of much scientific work, it wou
indeed be remarkable if, for these newer drugs, studied for only a few years, v
had already established how and where they act. There is even the disturbi
possibility that our understanding of how a drug, by chemical and physiologic
means, produces or alleviates an abnormal thinking process must await o
understanding of the chemistry and physics of normal thought. The importa
thing is that all of us have not only theories, of which the Greeks had an abu
dance, but we have laboratories, as well, and investigators in these laboratori
are busily at work.

Such important and provocative data are presented in these pages that
find it difficult to believe that many of the correlations and associations d
scribed are mere coincidence. I feel strongly that these data are trying to te
us a story, and I am happy that so many of us appear to be listening.

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